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PERSISTENCE OF ANTIBIOTICS IN LIQUID HOG MANURE APPLIED TO CROPLAND AND THEIR TRANSPORT IN SURFACE RUNOFF

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Persistence of Antibiotics in Liquid Hog Manure Applied to Cropland and their Transport in Surface Runoff

by

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Summary

Due to the proliferation of confined animal feeding operations, manure management has become an issue with respect to water quality both in terms of safety of drinking water and in terms of protecting and conserving aquatic life in sensitive ecosystems. The key objectives in this study were to determine the extent to which two antimicrobials, used to prevent post-weaning diarrhoea in pigs, persist in liquid swine manure during storage and following application to cropland, and to assess the potential for their transport in surface runoff from treated land into surface waters and for leaching to shallow ground water.

Technical Report

Graduate Student: The graduate student working on this project, Sandra Kuchta, is enrolled in the College of Graduate Studies and Research through the Toxicology Centre, University of Saskatchewan. She will defend her thesis research in 2007.

Introduction

Swine, poultry and cattle are increasingly being produced in large confined animal feeding operations. With such production, antimicrobials are administered, regardless of the health of the animals, to prevent disease and to promote growth by increasing the rate of weight gain. Since many antimicrobials are poorly absorbed through the gut, they can be excreted up to 80% or more in the feces and urine. Consequently, because of antimicrobials present in the manure, the application of manure as a nutrient source to crop and pasture land can be a potential source of contamination of surface and ground water via surface runoff and leaching, respectively.

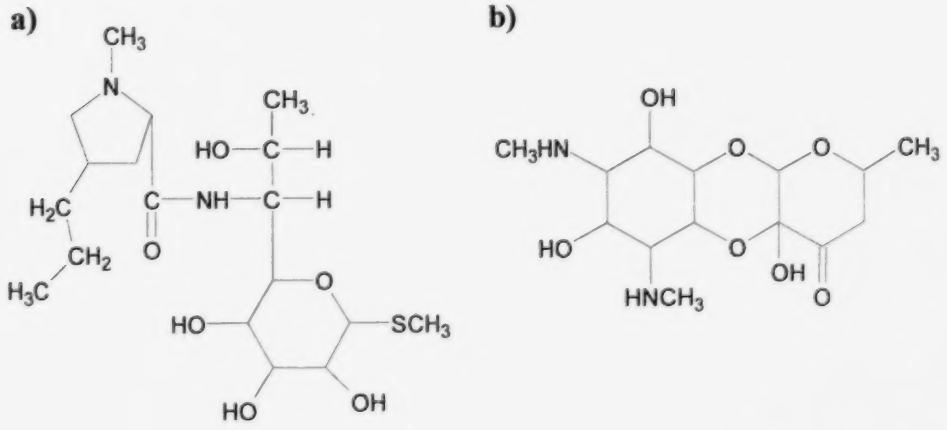
Recently, antimicrobials used in the livestock industry have been detected in surface waters in Canada (Forrest et al., 2006), the United States (Kolpin et al., 2002; Lindsay et al., 2001) and Europe (Calamari et al., 2003; Hirsch et al., 1999). Contamination of surface and ground waters by veterinary antimicrobials is a cause for concern because it may accelerate the development of antimicrobial-resistant bacteria. Although antimicrobials administered to humans are generally not

the same as those given to animals, their structures may be similar enough that veterinary antimicrobials can cause resistance to those used to treat humans. For example, Khatchatourians (1998) showed that when streptococci and staphylococci developed resistance to tylosin, a common animal feed additive, they also developed a resistance to erythromycin used in human medicine.

In 2002, over 28 million swine were marketed in Canada, of which almost 2 million were marketed in Saskatchewan. The export market, which accounts for 35% of the total production, annually generates over $ 1 billion in revenue. Antimicrobials are administered to swine to control gastrointestinal infections. Such control is critical to the industry because gastrointestinal infections can result in reduced growth rate, decreased food conversion, and mortality.

Two antimicrobials, lincomycin and spectinomycin (Figure 1a and 1b) are administered in the prairie region of Canada as a mixture to swine for the prevention and control of post-weaning diarrhoea. The possibility that these and other antimicrobials, following land application of manure, will be transported to surface and ground waters depends, in part, on the extent to which they persist in the stored manure and in the manure-applied soil.

Figure 1. Chemical structure of (a) lincomycin and (b) spectinomycin



of manure were collected during manure application by the manure applicator operators and analyzed for antimicrobial content.

Antimicrobials

At PSCI and RLSF, lincomycin and spectinomycin are administered as a mixture to weanling pigs to prevent post-weaning diarrhea until body weight increases from 6 to 35 kg. During this period, the pigs are housed in the nursery part of the barn and are administered the L-S® 20 Premix (Pfizer Animal Health) with their feed. This premix contains 22 g of lincomycin hydrochloride (the hydrochloric acid salt of lincomycin) and 22 g of spectinomycin sulfate (the sulfuric acid salt of spectinomycin) per kilogram of premix.

Study Sites and Manure Applications

Study sites consisted of crop and pasture land adjacent to the PSCI and RLSF swine barns. In some cases, study sites consisted of multiple fields, and each study site had a quarter section of land that was not manure applied and served as a control site. For all fields to which liquid swine manure was injected, a Coulter disc applicator (Bourgault Industries, St. Brieux, SK, Canada) (Photo 1) was used.

Photo 1 Photo 2

At the PSCI barn, liquid swine manure was contained in a single storage lagoon which was enclosed with a floating plastic cover to reduce odours. Two lagoons were utilized at the RLSF barn such that, when the primary lagoon was full, the liquid component of the manure overflowed into the secondary lagoon. Neither lagoon was covered. Manure from the primary lagoon was applied by injection, whereas the liquid component from the secondary lagoon was applied using a centre pivot (Photo 2).

All arrangements for manure applications and for land to which manure would be applied were made by barn personnel. They also provided information on rates of application of liquid swine manure to crop and pasture land. Samples of manure were collected by the manure applicator operators during manure application at PSCI study site 1, PSCI study Site 2 and at RLSF study site 4. The number of samples collected for each application ranged from two to six.

PSCI Study Sites

PSCI study site 1 (2003 and 2004): This study site was being used in an ongoing study to determine the impact of the application of liquid swine manure to cropland on surface and ground water quality with respect to nitrogen and phosphorus. It consisted of four quarter sections (64.75 ha each) of land that were farmed by the same producer. Two of the quarter sections were divided into halves such that one half of each quarter section was treated with liquid swine manure in the fall of 2003 at approximately 60,000 L ha^{-1} and the other half with approximately 95,000 L ha^{-1}. A single watershed which drained into a depression was defined in each half of each quarter section and each watershed was instrumented with a single piezometer located at the center of the depression. Liquid swine manure was last applied to these two quarter sections in fall 2001. A third quarter section, to which liquid-swine manure was last applied in fall 2002, acted as a control watershed and was similarly instrumented with a piezometer. The fourth quarter section, to which liquid swine manure had not been applied in the previous five years, was also used as a control, but no piezometer was installed. All quarter sections were cropland with either canary seed or wheat stubble. None of these quarter sections contained wetlands.

1.4.2 PSCI study Site 2 (2004 and 2005): This site consisted of two quarter sections, one that had manure applied and the other that did not. Both quarter sections were farmed by the same producer and were cropland with canola stubble. Liquid swine manure was injected into one quarter section in the fall of 2004 at rate of approximately 78,000 L ha^{-1}. Manure had not been previously applied to these quarter sections in the past five years. There were two ephemeral wetlands on the treated quarter section, and one on the control quarter section.

1.5 RLSF Study Sites

In 2004, study sites 1, 2 and 3 were farmed by the same producer.

1.5.1 RLSF study site 1 (2004): This field was approximately 32 ha of summer fallow and 48 ha of cereal crop stubble. Manure from the primary lagoon was injected into both the summer fallow and stubble portions of this quarter section at a rate of approximately 79,000 L ha^{-1}. There were no wetlands on this site, but there were seven areas on the summer fallow portion of the field where snow melt runoff accumulated and complete infiltration did not occur until mid April.

1.5.2 RLSF study site 2 (2004): This site consisted of two quarter sections (one with canola stubble and one with cereal stubble) with three ephemeral wetlands located within the canola stubble. Liquid swine manure was applied in fall 2003 from the secondary lagoon to these quarter sections using the centre pivot at a rate of approximately 3,000 L ha^{-1}.

1.5.3 RLSF study site 3 (2005): This was the only study site to receive a spring application of liquid swine manure. This site consisted of two quarter sections (canola stubble): one with two ephemeral wetlands and the other with three. Manure was injected in the spring of 2004 at a rate of approximately 90,000 L ha^{-1}.

1.5.4 RLSF study site 4 (2005): This site consisted of three quarter sections of pasture land farmed by a different producer. One quarter section served as the control, the other two quarter sections had liquid-swine manure injected at rates of approximately 88,000 and 110,000 L ha^{-1}, respectively.

There was a dugout (small constructed reservoir) situated on each quarter section. Since they derive their water from surface runoff, all were monitored for antimicrobial content. Four ephemeral wetlands on one of the manure-treated quarter sections were also monitored.

Antimicrobial Use at PSCI and RLSF

Depending on lagoon size and the barn operation, manure may be applied once or twice a year, usually in the spring and/or the fall. Consultation with barn personnel at both study site locations (PSCI and RLSF) indicated that the PSCI lagoon was emptied once in 2003 (fall) and once in 2004 (fall), whereas the RLSF lagoons were emptied in both the spring and fall of both years. Information on the rates of manure application was also obtained from barn personnel each year.

Barn personnel also provided average amounts of lincomycin and spectinomycin used per month in their respective barns. Lincomycin and spectinomycin were administered together as a premix (LS-20, Pfizer Animal Health) in the feed at both barns. At the RLSF barn, approximately twenty-four times more lincomycin was used compared to spectinomycin (2.86 and 0.12 kg $month^{-1}$, respectively). At the PSCI barn, the use of lincomycin and spectinomycin was reported as approximately equal (1.07 and 0.9 kg $month^{-1}$, respectively).

1.7 Solids Content of Liquid Swine Manure

The solids content was lowest in the liquid swine manure from the weanling pigs in the nursery part of the PSCI barn and increased from 1.3 to 3.2% over a 5-week period as the pigs became older (Table 1). The highest solids content was from older pigs in the grower-finisher part of the barn where the solids content varied from 11 to 25%. This variation may reflect, in part, differences in the amount of wash water present in the manure pits from which the samples were taken. The liquid manure applied to cropland from lagoons had solids contents which varied from approximately 10 to greater than 20%. The solids content in the lagoon manure generally decreased during manure application. This most likely reflects the practice of adding water to the lagoons to suspend manure solids during the pumping of the manure to the applicator in the field.

Table 1. Solids content of liquid hog manure samples collected from the PSCI and RLSF study sites for the fortification and simulated manure storage studies, during the excretion study and during fall manure applications to cropland.

Sample	Barn	Date collected	Solids content (%)
Grower-Finisher part of barn			
Fortification sample	PSCI	Jan-04	11.3 ± 0.9
Simulated manure storage container 1	PSCI	Jul-05	25.0 ± 1.6
Container 2	PSCI	Jul-05	13.2 ± 1.3
Nursery part of the barn			
Week 1 of excretion study	PSCI	Jun-05	1.3
Week 2	PSCI	Jun-05	1.8
Week 3	PSCI	Jun-05	2.6
Week 4	PSCI	Jul-05	3
Week 5	PSCI	Jul-05	3.2
Lagoon (PSCI)			
Fall application sample 1	PSCI	Oct-03	11
Sample 2	PSCI	Oct-03	10
Sample 3	PSCI	Oct-03	10
Sample 4	PSCI	Oct-03	9
Lagoon (PSCI)			
Fall application sample 1	PSCI	Oct-04	22
Sample 2	PSCI	Oct-04	20
Sample 3	PSCI	Oct-04	19
Sample 4	PSCI	Oct-04	17
Sample 5	PSCI	Oct-04	17
Sample 6	PSCI	Oct-04	17
Lagoon (RLSF)			
Fall application sample 1	RLSF	Oct-04	26
Sample 2	RLSF	Oct-04	24

Objective 1 - *To develop analytical methods to detect residues of lincomycin and spectinomycin in liquid swine manure, manure-treated soil, and in surface and ground waters.*

Method Development

Results: Analytical methods have been developed to determine concentrations of the antimicrobials lincomycin and spectinomycin in water, liquid swine manure (solids and liquid components) and

manure-treated soil. For the liquid component of the swine manure, surface water and ground water, solid-phase extraction (Photo 3) and liquid chromatography interfaced with mass spectrometric detection (LC-MS-MS) were used for quantification and confirmation. Pressurized liquid extraction (Photo 4) followed by solid-phase extraction clean-up was used for the solids component of the liquid swine manure and the manure-treated soil. The sensitivity of the method for lincomycin was much higher than that for spectinomycin. The limits of quantification in surface and ground water were 0.008 $\mu g\ L^{-1}$ and 0.2 $\mu g\ L^{-1}$, respectively, whereas those in liquid swine manure (both the solids and liquid components) and manure-treated soil were 0.04 and 6.0 $\mu g\ L^{-1}$, respectively. The analytical method developed for surface water and liquid component of the swine manure has been published (Peru et al. 2006).

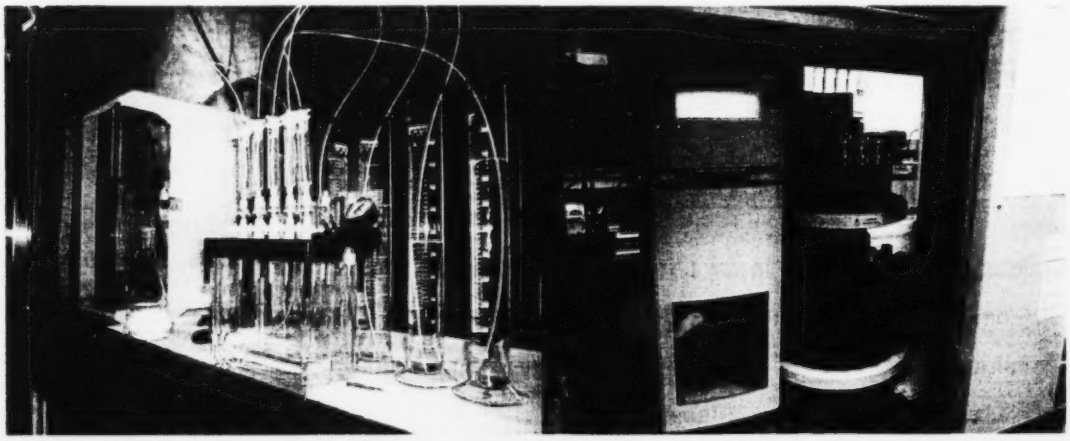


Photo 3

Photo 4

Additional method development: To facilitate future studies, methods were developed for the extraction and analysis of the veterinary antimicrobials chlortetracycline and sulfamethazine in surface water and for the determination of chlortetracycline residues in solid beef cattle manure.

Objective 2 - *To quantify, by analysis of liquid swine manure, the extent of excretion of these antimicrobials used to prevent post-weaning diarrhoea in swine.*

Antimicrobials Excretion

Weanling pigs (Photo 5) were administered a 5-phase diet in which lincomycin and spectinomycin were added to the feed as a premix (L-S 20, Pfizer Animal Health) in phases 1 to 4 (sampling weeks 1 to 4) and no antimicrobial was added during phase 5 (sampling week 5). The premix contained 22 g of lincomycin hydrochloride and spectinomycin sulfate per kilogram of premix. The amount of premix in the feed during phase 1 was confidential, the feed contained 0.1% premix in phases 2, 3 and 4, and no premix was added in phase 5. The total amount of feed consumed by the 270 weanling pigs during the study period was 8.04 tonnes. Based on lincomycin and spectinomycin being present in the premix as their respective hydrochloride and sulfate salts, the total amounts administered (phases 2-4) to the weanling pigs during the excretion study were approximately 162.1 and 136.6 g, respectively. The 270 pigs in the study room in the nursery part of the barn produced approximately 17,000 L of liquid manure during the 5-week period. The average solids content of the liquid manure produced was 2.4%. Based on the amount of antimicrobials administered to the animals (assuming 100% excretion) and the volume of the manure produced, maximum concentrations expected in the total volume of liquid swine manure would be 9,535 $\mu g\ L^{-1}$ of lincomycin and 8,035 $\mu g\ L^{-1}$ of spectinomycin. Because the amounts of the antimicrobials administered in phase 1 were not known, the final antimicrobial concentrations in the liquid swine manure are somewhat underestimated.

Manure samples were collected weekly for 5 weeks. Manure, in each pit was sampled by the following procedure: floor slats at three locations in the hallway were removed (Figure 3). A power drill, equipped with a paint mixer and attached to a 1.5-m long pipe, was lowered into the pit to thoroughly mix the manure in the sampling area. At each sampling location, a mixed liquid manure subsample (~5 L) was scooped from the pit with a long-handled ladle and the three subsamples were combined in a 19-L plastic pail. This composite sample (~15 L) was mixed again with the power drill and a 1-L subsample was transferred to a sample bottle (1-L Nalgene Teflon FEP). At each sampling time, a manure sample for residue analysis was taken from each pit.

Figure 3. Layout of Nursery Room and Sampling Locations at the PSCI Barn

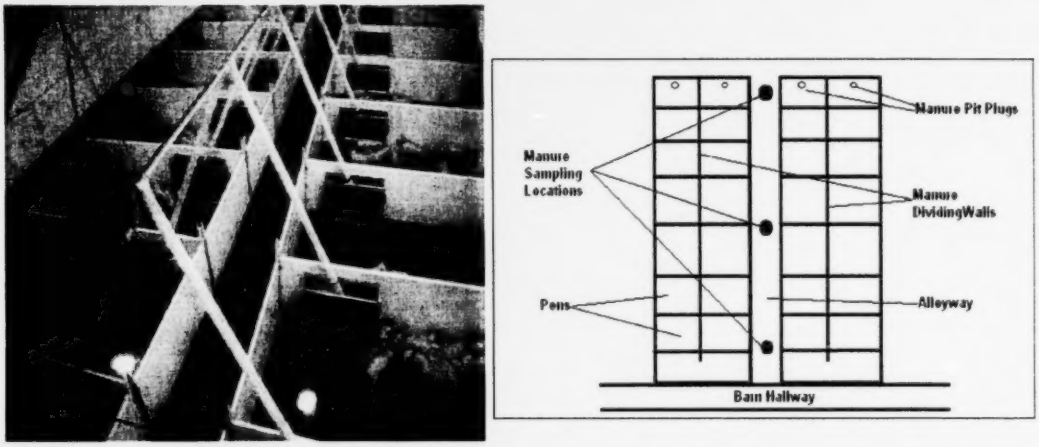


Photo 5

Results: Lincomycin and spectinomycin concentrations detected in the liquid swine manure collected on the last sampling day (sampling week 5) were 1,990 μg L^{-1} and 135 μg L^{-1}, respectively (Table 2). The ratio of lincomycin to spectinomycin detected in the liquid swine manure was approximately 14.1:1 rather than that (1.19:1) expected from the ratio in the L-S 20 Premix.

Concentrations of both lincomycin and spectinomycin generally increased with time and then decreased. Lincomycin concentrations peaked during week 4 whereas those for spectinomycin peaked during week 3 (Table 2). The concentrations of lincomycin and spectinomycin present in the liquid manure at the termination of the study represented amounts equivalent to 20.9 and 2.4%, respectively, of the maximum expected concentrations.

Liquid swine manure samples collected from the study room in the nursery part of the barn contained, on average, 2.4% solids (from data in Table 1). Separate extraction of the solids and liquid components of the manure showed that, in all samples, lincomycin was present in both manure components whereas spectinomycin was present only in the liquid component. Lincomycin was also mainly present in the liquid component with a maximum of 1.3% in the solids component.

Table 2. Concentrations of lincomycin and spectinomycin detected in liquid manure from weanling pigs collected from the study room in the nursery part of the barn.

Sample Week	Nursery Study Room Manure	
	Lincomycin ($\mu g\ L^{-1}$)	Spectinomycin ($\mu g\ L^{-1}$)
1	2,730	192
2	3,380	253
3	3,180	268
4	3,450	169
5	1,990	135

Objective 3 - ***To determine the extent to which both antimicrobials persist in the manure during simulated storage and in soil following manure injection into cropland or pasture land.***

The PSCI lagoon was enclosed with a floating plastic cover to reduce odours. To simulate a covered lagoon in the laboratory, fortified control liquid manure was placed in stainless steel containers (20 L) with fitted stainless steel lids and maintained at room temperature (approximately 21°C) in a fume hood.

To determine recovery of lincomycin and spectinomycin, control manure was fortified at 50 and 500 $\mu g\ L^{-1}$, respectively, to ensure quantifiable amounts of both antimicrobials in the solids component of the liquid manure. The fortified manure samples were maintained at -40°C until extraction and analysis. Mean recoveries of lincomycin from the fortified liquid manure (liquid and solids components combined) was 71 ± 14% (n = 4) with proportions in the liquid and solids components being 81 ± 11% and 19 ± 11%, respectively. Mean recoveries of spectinomycin from the fortified liquid manure was 58 ± 16% (n = 4) with proportions in the liquid and solids components being 71 ± 18% and 29 ± 18%, respectively.

Persistence during Simulated Manure Storage

Two 15-L samples of control manure from pigs not administered antimicrobials were collected and fortified at 40 and 400 μg L^{-1} with lincomycin and spectinomycin, respectively (Photo 6). The fortified control manure contained more solids component than nursery manure, the solids content in containers 1 and 2 being 25% and 13%, respectively (Table 1). Subsamples (100 mL) of the fortified manure were collected weekly for five months and then maintained at -40°C until extraction. Sub-samples of the manure collected on day 0, before fortification, were analyzed and no lincomycin or spectinomycin was detected.

Photo 6

Results: *Lincomycin* - The concentrations of lincomycin detected in the liquid manure sampled 4 hours after fortification (day 1) were 60.28 μg L^{-1} and 46.51 μg L^{-1} in containers 1 and 2, respectively (Table 3a and 3b). Concentrations of lincomycin in both containers were higher than the target concentration of 40 μg L^{-1}. By day 3, the corresponding concentrations of lincomycin had decreased to 42.54 μg L^{-1} and 39.64 μg L^{-1}. From days 6 to 154 the concentration of lincomycin remained essentially constant in both containers (Figure 4a and 4b).

The proportion of lincomycin associated with the solids component of the liquid manure was much higher for the control manure samples than for the manure from the pits in the nursery part of the barn. This may be a function of the larger solids content in the control manure samples. The

Tables 3a and 3b. Concentrations of lincomycin ($\mu g\ L^{-1}$) in the solids and liquid components during simulated storage of fortified liquid swine manure.

Table 3a - Container 1

Day	Liquid component	Solids component	Total	Percent from solids (%)
1	37.7	22.6	60.3	37.5
3	25.0	17.6	42.5	41.3
6	12.4	7.8	20.2	38.8
10	16.5	20.5	37.1	55.4
15	6.2	31.2	37.5	83.4
27	11.3	21.6	33.0	65.6
41	16.4	18.9	35.4	53.6
62	18.6	9.9	28.5	34.7
69	15.5	20.5	36.1	56.9
90	22.0	1.2	23.2	5.2
107	24.5	11.6	36.1	32.3
154	23.9	7.6	31.5	24.2
		Mean	31.8†	44.1
		Std Dev	6.1	20.5

Table 3b - Container 2

Day	Liquid component	Solids component	Total	Percent from solids (%)
1	41.2	5.3	46.5	11.4
3	34.5	5.1	39.6	12.9
6	33.2	6.5	39.7	16.5
10	30.9	4.9	35.8	13.8
15	28.1	5.9	33.9	17.3
27	32.1	2.7	34.8	7.8
41	30.7	7.1	37.8	18.8
62	18.9	12.3	31.2	39.4
69	17.9	10.9	28.8	37.7
90	25.1	15.1	40.2	37.5
107	25.6	ns	-	-
154	24.0	ns	-	-
		Mean	35.3†	21.3%
		Std Dev	4.0	12.1%

*Solids component extract was lost due to instrument malfunction

†Mean concentration is for values from day 6 to 154

Figure 4a. Concentration of lincomycin versus time detected in the solids and liquid components collected from container 1 during simulated liquid swine manure storage.

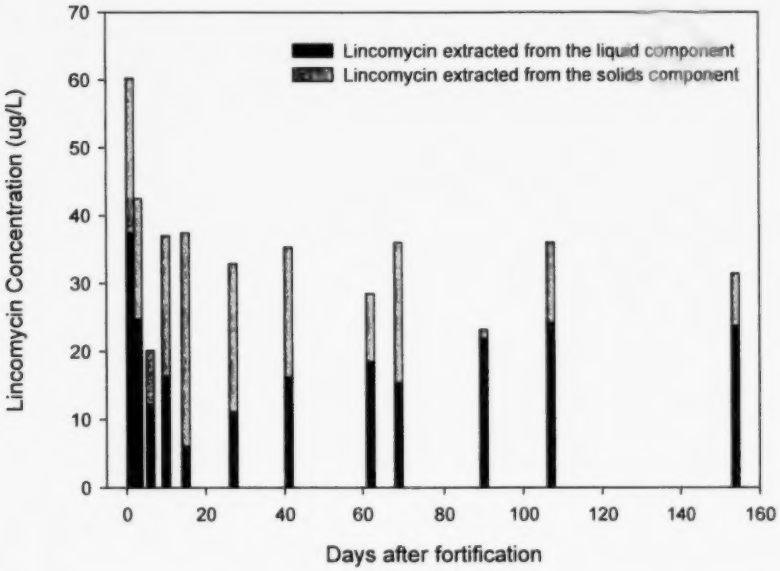


Figure 4b. Concentration of lincomycin versus time detected in the solids and liquid components collected from container 2 during simulated liquid swine manure storage.

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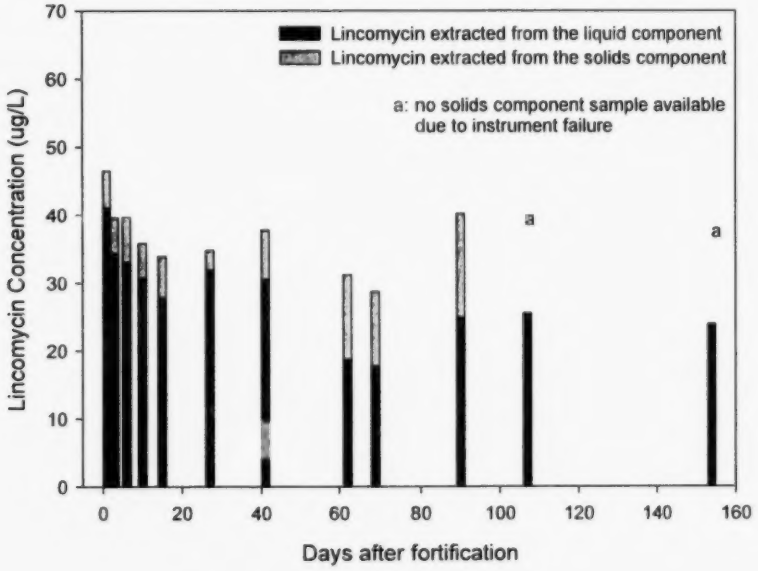
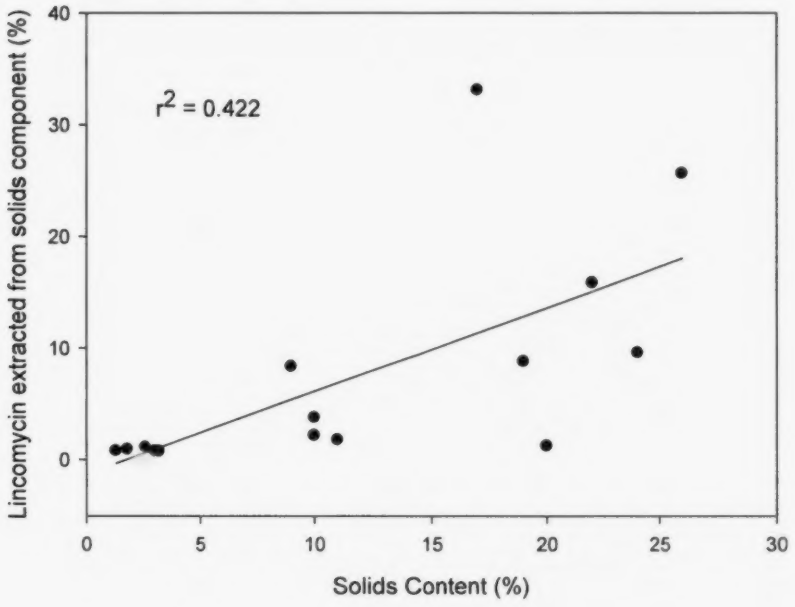


proportion of lincomycin extracted from the solids component in container 1 (25% solids) was 44.1 ± 20.5% whereas that extracted from solids component in container 2 (13% solids) was 21.3 ± 12.1% (Tables 3a and 3b) suggesting that the amount of lincomycin associated with the solids component was related to the solids content of the liquid manure. Plotting the proportion of lincomycin associated with the solids component of the nursery manure and manure from lagoons applied to the PSCI and RLSF study sites against the solids content of the liquid manure resulted in a straight-line plot which supported this relationship (Figure 5). This correlation, which shows an increasing lincomycin content in the solids component of the manure as the solids component increases, is not strong ($r^2 = 0.422$). However, the weakness in the correlation may, in part, reflect the difficulty in obtaining homogeneous subsamples for analysis (that is, subsamples with a consistent solids content), especially as the solids content of the manure increases.

Figure 5. Relationship between the solids content of the liquid-swine manure and the amount of lincomycin extracted from the solids component of the nursery manure and the lagoon manure applied to the PSCI and RLSFD study sites.



Results: *Spectinomycin* - The spectinomycin concentrations detected in samples collected four hours after fortification on day 1 (292 $\mu g\ L^{-1}$ and 231 $\mu g\ L^{-1}$ in containers 1 and 2, respectively) were lower than the target fortification level of 400 $\mu g\ L^{-1}$ (Tables 4a and 4b). Like lincomycin, the concentration of spectinomycin had rapidly decreased by day 3 and then remained essentially constant from days 6 to 107 in both containers (Figures 6a and 6b).

In each container, there was one sub-sample where spectinomycin was not detected in either the solids or liquid components (container 1, day 90 and container 2, day 15), but it was detected in sub-samples collected before and after these days (Figures 6a and 6b). Spectinomycin was not detected in the sub-samples collected from both containers on day 154 in either the solids or liquid components. There was also a sub-sample from container 1 where spectinomycin was not detected in the liquid component (day 41) and one from container 2 in which spectinomycin was not detected in the solids component (day 90). Since it was not clear why this happened, the extraction and analysis of these sub-samples were repeated, however, the same results were obtained. The concentration pattern in Figures 6a and 6b suggest that it is likely that spectinomycin was present in these sub-samples (as well as those from both containers on day 154), despite being non-detected. Spectinomycin may not have been detected either because the pressurized liquid extraction was not effective or there was suppression of spectinomycin ionization during mass spectral analysis of these sample extracts.

Like lincomycin, the proportion of spectinomycin associated with the solids component of the liquid manure was much higher for the manure samples from the grower-finisher part of the barn than that from the nursery part of the barn. No spectinomycin was detected in the solids component of the nursery manure (mean solids content of 2.4%) and this may be a function of the 12-fold smaller proportion of spectinomycin in the manure compared to lincomycin. However, an average of 18.6 ± 2.3% and 19.0 ± 6.6% of spectinomycin was extracted from the solids component of containers 1 (25% solids) and 2 (13% solids), respectively (Tables 4a and 4b). Since spectinomycin was not detected in the nursery manure or the lagoon manure applied to the PSCI and RLSF study sites, it was not possible to establish whether or not a correlation existed between the proportion associated with the solids component of the manure and the solids content of the manure.

Tables 4a and 4b. Concentrations of spectinomycin (μg L^{-1}) in the solids and liquid components during simulated storage of fortified liquid swine manure.

Table 4a - Container 1

Day	Liquid component	Solids component	Total	Percent from solids (%)
1	234	58.4	292	20.0
3	98.7	19.2	118	16.3
6	78.9	12.9	91.7	14.0
10	70.3	15.3	85.6	17.9
15	62.2	15.0	77.2	19.4
27	57.8	13.2	71.1	18.6
41	nd	13.2	13.2	100.0
62	52.3	13.6	65.9	20.6
69	55.8	14.7	70.5	20.8
90	nd	nd	0.0†	0.0
107	52.5	13.2	65.7	20.1
154	nd	nd	0.0†	0.0
		Mean	64.2‡	22.3
		Std Dev	23.5	25.6

Table 4b - Container 2

Day	Liquid component	Solids component	Total	Percent from solids (%)
1	150	81.4	232	35.1
3	79.5	14.0	93.4	14.9
6	60.0	12.0	71.9	16.6
10	53.9	11.5	65.4	17.6
15	nd	nd	0.0†	0.0
27	50.5	11.3	61.8	18.2
41	57.4	12.3	69.8	17.7
62	55.9	10.5	66.4	15.8
69	52.0	9.6	61.6	15.6
90	50.6	nd	50.6	100.0
107	56.6	ns*	56.6	100.0
154	nd	ns	-	-
		Mean	65.0‡	32.0
		Std Dev	7.0	34.6

*Solids component extract was lost due to instrument malfunction

†Value not included in calculation of the mean

‡Mean concentration is for values from day 6 to 154

Figure 6a. Concentration of spectinomycin versus time detected in the solids and liquid components collected from container 1 during simulated liquid swine manure storage.

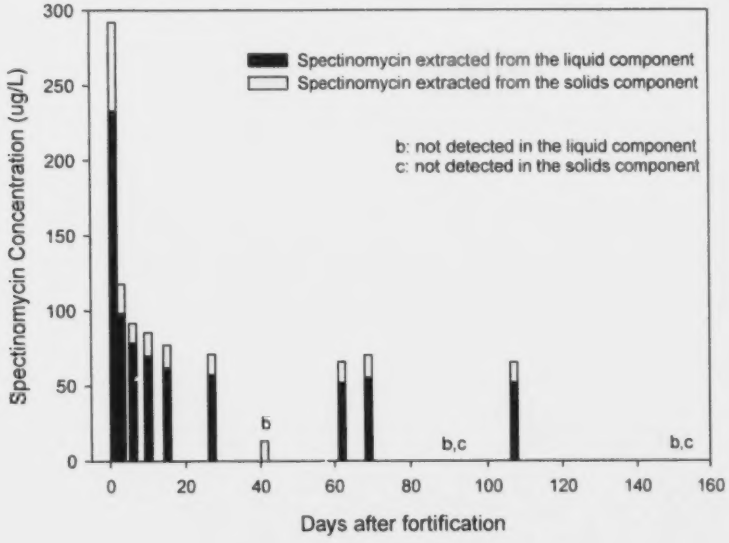
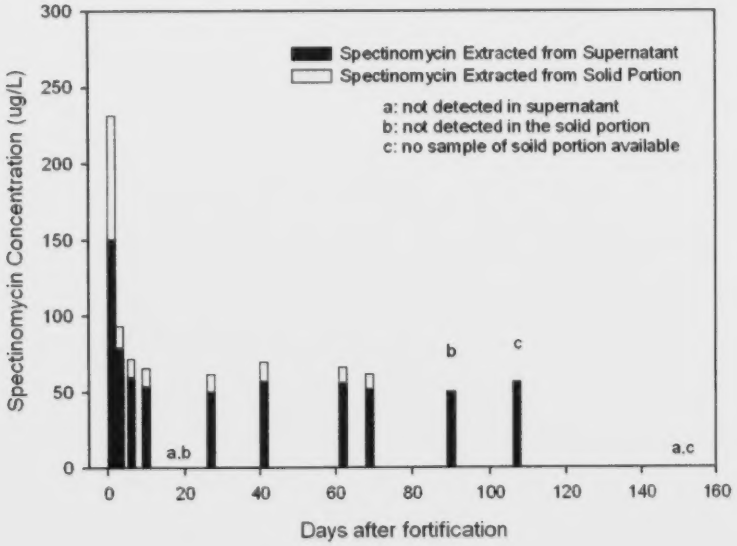


Figure 6b. Concentration of spectinomycin versus time detected in the solids and liquid components collected from Container 1 during simulated liquid swine manure storage.



Persistence in Soil

In the fall of 2003 and 2004, soil samples were collected from all fields at PSCI Study Sites 1 and 2, respectively, before and after manure application. After snow melt runoff the following spring, soil samples were collected monthly until freeze-up in the fall of 2004 and 2005, respectively. In each field, soil samples were collected at upper, mid and lower slope positions as follows: a metal template (1-m long x 5-cm wide x 5-cm deep) was pounded into the soil such that it encompassed a minimum of three injection furrows (Photo 7). The soil (approximately 2,500 cm^3) contained within the template was collected with a narrow trowel. Soil samples were placed in freezer bags and maintained at -40°C until extraction and analysis using the methodology described in objective 1.

Photo 7

Results: *Manure analysis* - Spectinomycin was not detected in any of the liquid manure samples collected by the manure applicator operators while applying liquid manure from both the PSCI and RLSF lagoons to their respective study sites. In contrast, lincomycin was detected in all manure samples collected by the applicator operators. Lincomycin concentrations in liquid manure applied to the PSCI study sites from the PSCI lagoon in the falls of 2003 and 2004 ranged from 105 to 179 μg L^{-1} (mean = 132 μg L^{-1}) and 45.8 to 231 μg L^{-1} (mean = 122 μg L^{-1}), respectively (Table 5). Concentrations in manure applied to the RLSF study site from the RLSF lagoon in fall 2004 ranged from 25.1 to 38.5 μg L^{-1} (mean = 31.8 μg L^{-1}). (Note that results from the analysis of the individual manure samples are included in Appendix A).

Table 5. Manure application rates and volumes and lincomycin concentrations in the applied manure

Study Site	Manure application rate ($L\ ha^{-1}$)	Total volume of manure applied (ML)	Theoretical maximum concentration ($\mu g\ L^{-1}$)		Mean Lincomycin Concentration ($\mu g\ L^{-1}$)
			Lincomycin	Spectinomycin	
PSCI 1 (2003)	60,000 95,000	3.9 6.1 5.0*	855	720	132 (n = 4)
PSCI 2 (2004)	78,000	5.0 10.0*	855	720	122 (n = 4)
RLSF 1 (2004)	79,000	6.3 6.7*	2,640	112	ns
RLSF 2 (2004)	3,000	3.8 9.2*			ns
RLSF 3 (2005)	90,000	11.5 1.5*	2,640	112,307	ns
RLSF 4 (2005)	88,000 110,000	5.7 3.6 3.7*			31.8 (n = 2)

ns: no sample was collected by the manure applicator operator
*manure applied to non-study site fields

Results: *Soil analysis* - Lincomycin and spectinomycin were not detected in soil samples collected from the control watersheds. As expected, since spectinomycin was not detected in the liquid manure applied to the PSCI and RLSF study sites, spectinomycin was not detected in any soil samples collected from manure-treated sites. In contrast, immediately after manure application in the falls of 2003 and 2004, lincomycin was detected in all soil samples collected from the three slope positions on manure-treated sites in concentrations ranging from 200 to 1,595 $\mu g\ kg^{-1}$ (Tables 6a and 6b). Following spring snow melt runoff, lincomycin was detected in the soil until mid-June in 2004 and mid-July in 2005 and concentrations during these periods ranged from <0.04 to 964 $\mu g\ kg^{-1}$. At PSCI study site 2 in 2005, lincomycin was detected in soil only from the lower slope position; however, this pattern was not observed for the PSCI study 1 watersheds in 2004. In general, lincomycin concentrations tended to decrease after spring snow melt runoff through to mid-summer.

Table 6a. Lincomycin concentrations (μg kg^{-1}) detected in manure-treated soil samples (0-5 cm) collected at three slope positions (upper, mid and lower) of each watershed (WS) at PSCI study site 1. Two liquid manure application rates (60,000 and 95,000 L ha^{-1}) were used in fall 2003.

Sample Date	WS1 (60,000 L ha^{-1})			WS2 (60,000 L ha^{-1})			WS3 (95,000 L ha^{-1})			WS4 (95,000 L ha^{-1})		
(dd/mm/yyyy)	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower
03/10/2003	1396	1092	1020	925	462	934	876	376	1095	776	1595	879
28/04/2004	ns	ns	ns	824	nd	nd	964	nd	nd	655	942	nd
31/05/2004	321	nd	nd	756	nd	nd	646	nd	nd	303	786	nd
29/06/2004	nd	nd	nd	341	nd	nd	422	nd	nd	nd	256	nd
30/07/2004	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
28/08/2004	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
06/10/2004	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

ns: no sample collected
nd: not detected

Table 6b. Lincomycin concentrations detected in manure-treated soil samples (0-5 cm) collected at three slope positions (upper, mid and lower) adjacent to two ephemeral wetlands (W) and one depression (D) at PSCI study site 2. Liquid manure was applied at 78,000 L ha^{-1} in fall 2004.

Sample Date	W1 (78,000 L ha^{-1})			W2 (78,000 L ha^{-1})			D1 (78,000 L ha^{-1})		
(dd/mm/yyyy)	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower
06/10/2004	444	535	835	400	390	830	200	390	800
24/05/2005	nd	nd	780	nd	nd	590	nd	nd	740
21/06/2005	nd	nd	770	nd	nd	330	nd	nd	620
19/07/2005	nd	nd	90	nd	nd	40	nd	nd	100
23/08/2005	nd	nd	nd	nd	nd	nd	nd	nd	nd
20/09/2005	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detected

Objective 4 - *To assess the potential for their transport in surface runoff from manure-treated land into surface water bodies using simulated rainfall runoff experiments and by monitoring snow melt runoff.*

Simulated Rainfall Runoff

Simulated rainfall runoff experiments were carried out within a week of manure application at PSCI study sites 1 and 2 in the falls of 2003 and 2004, respectively. At PSCI study site 1, rainfall simulation experiments were carried out in the defined watersheds at upper, mid and lower slope positions. At PSCI study site 2, rainfall simulation experiments were similarly carried out at three sites within the manure-treated quarter section.

Rainfall simulator: Simulated rainfall was produced from a 1.5-m boom equipped with two nozzles positioned 50 cm apart. The boom was mounted on top of a 1.5-m wide x 1.5-m long x 2-m high frame constructed from plastic pipe (Photo 8). Tarps were attached to each of the four sides of the frame to minimize wind interference of the simulated rainfall. Water, from a municipal well near the PSCI study sites, was pumped at approximately 2 L min^{-1} to the boom via a hose from a 3,400-L tank mounted on the bed of a truck.

Photo8

The tarp-covered frame was placed over a 1-m x 1-m plot of land which encompassed at least three injection furrows on the manure-treated sites. In order to define the 1-m by 1-m simulation plot,three metal sheets (1-m long by 10-cm high) were hammered into the soil such that approximately 5 cm of each sheet remained above the soil surface (Photo 9). On the fourth side of the plot, a triangular metal tray (flume) was placed approximately 1 cm below ground level in order to collect simulated rainfall runoff from the plot. Rain gauges were placed in the center and at the back of the 1-m by 1-m plot in order to estimate the amount of simulated rainfall applied to the study plot during each simulation (Photo 9). A hole, dug at the tip of the triangular tray, permitted collection of runoff water from the plot into 1-L amber glass bottles (Photo 10).

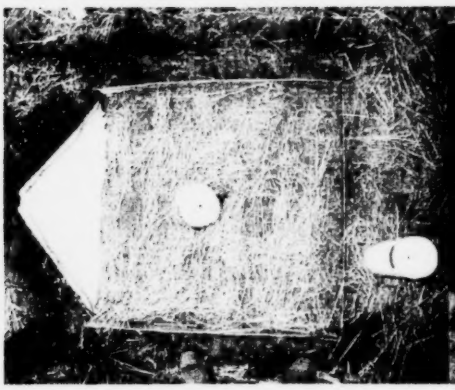


Photo 9

Photo 10

Rainfall simulation: Prior to the start of each experiment, tent pegs were used to fasten the lower edges of each tarp to the ground to prevent the simulator from being blown over by the wind. After simulated rainfall was begun and runoff from the plot began to flow over the triangular metal tray, runoff samples (1 L) were collected beginning at 5, 15, and 25 minutes after runoff began. Immediately after collection of the 25-min sample, the pump supplying water to the boom was turned off. However, runoff was still collected so that the total runoff volume could be measured. The runoff water samples were placed on ice in a cooler and transported to the laboratory where they were maintained at 4 $^{\circ}$C until extraction.

Results: *PSCI study site 1* -In fall 2003, simulated rainfall runoff was difficult to achieve because the soil was very dry and the water preferentially infiltrated into the soil. Consequently, simulated rainfall runoff samples were only collected from the control watershed and one manure-treated watershed (60,000 L ha^{-1}). Lincomycin and spectinomycin were not detected in any runoff samples from the control watershed. Lincomycin was detected (0.027 to 0.160 µg L^{-1}) in 100% of the runoff samples collected from the manure-treated watershed (Table 7). As expected, because spectinomycin was not detected in the manure applied to this site, it was not detected in any of the runoff samples collected from the manure-treated field.

Because of the limited number of simulated rainfall runoff experiments achieved in fall 2003 due to the dry conditions, experiments were repeated at PSCI study site 1 in May 2004 after snow melt runoff water in the depressions had infiltrated or evaporated. Lincomycin and spectinomycin were not detected in any simulated rainfall runoff samples from the control watershed. Spectinomycin was not detected in any simulated rainfall runoff samples collected from the manure-treated watersheds.

In the simulated rainfall runoff samples collected from the watersheds with the lower manure application rate (60,000 L ha^{-1}), lincomycin was detected in 55% of the samples with a maximum concentration of 0.075 µg L^{-1} (Table 7). Lincomycin concentrations in runoff samples from the watersheds with the higher manure application rate (95,000 L ha^{-1}) were generally higher than those detected in samples from watersheds that received the lower manure application rate (60,000 L ha^{-1}). At the higher manure application rate, the maximum concentration of lincomycin detected was 0.507 µg L^{-1}, and the frequency of detection was 83% (Table 7).

In both the fall 2003 and May 2004 simulated rainfall runoff experiments, lincomycin concentrations were generally highest in the sample collected at 5 minutes and then progressively decreased in the 15- and 25-minute samples (Table 7, Figure 7). (Note that similar Figures for all of the manure-treated watersheds at PSCI study site 1 are presented in Appendix B.) Lincomycin concentrations were lower in the spring experiments than those carried out in the fall on the same watershed, with

16 to 57% of the lincomycin detected in fall 2003 being detected in May 2004. There was no consistent pattern of lincomycin concentration in runoff water relative to slope position.

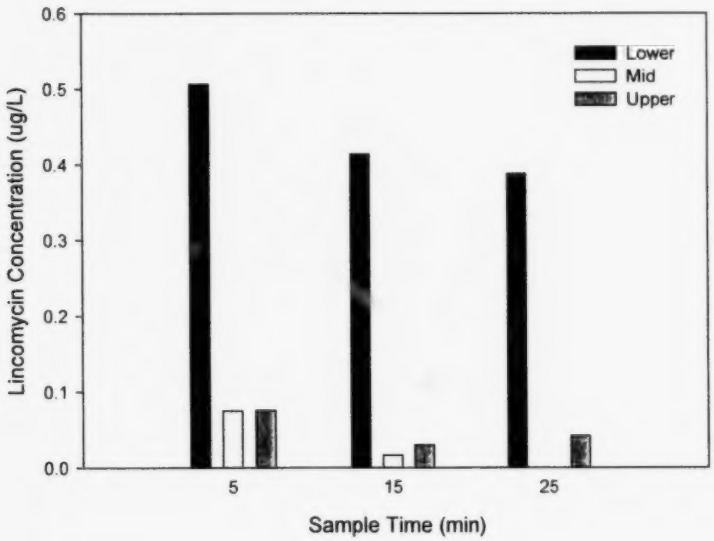
Table 7. Lincomycin concentrations detected in simulated rainfall runoff collected at three slope positions (upper, mid and lower) from watersheds (WS) at PSCI study site 1 treated with liquid swine manure in fall 2003 at two application rates (60,000 and 95,000 L ha^{-1}).

Slope Position	Time after simulated rainfall runoff began (min)	WS1 (60,000 L ha^{-1})	WS2* (60,000 L ha^{-1})	WS3* (95,000 L ha^{-1})	WS4* (95,000 L ha^{-1})	Control
		($\mu g\ L^{-1}$)	($\mu g\ L^{-1}$)	($\mu g\ L^{-1}$)	($\mu g\ L^{-1}$)	($\mu g\ L^{-1}$)
Fall 2003						
Lower	5	0.16	-	-	-	nd
	15	0.142	-	-	-	nd
	25	0.119	-	-	-	nd
Mid	5	-	-	-	-	nd
	15	-	-	-	-	nd
	25	-	-	-	-	nd
Upper	5	0.13	-	-	-	nd
	15	0.052	-	-	-	nd
	25	0.027	-	-	-	nd
Spring 2004						
Lower	5	0.047	0.042	0.507	0.021	nd
	15	nd	0.010	0.414	nd	nd
	25	0.019	nd	0.388	nd	nd
Mid	5	0.038	nd	0.075	0.051	nd
	15	0.02	nd	0.017	0.016	nd
	25	0.018	nd	nd	0.013	nd
Upper	5	0.075	nd	0.076	0.054	nd
	15	0.028	nd	0.030	0.022	nd
	25	0.013	nd	0.042	0.014	nd

*Due to dry conditions in fall 2003, it was not possible to carry out simulated rainfall runoff experiments on each watershed. Experiments on all watersheds were carried out in spring 2004 following snow melt runoff.

nd: not detected

Figure 7. Lincomycin concentrations (μg L^{-1}) detected in simulated rainfall runoff samples collected in spring 2004 at three slope positions (upper, mid, lower) from watershed 3 at PSCI study site 1 which had been treated with liquid swine manure in fall 2003 at 95,000 L ha^{-1}.



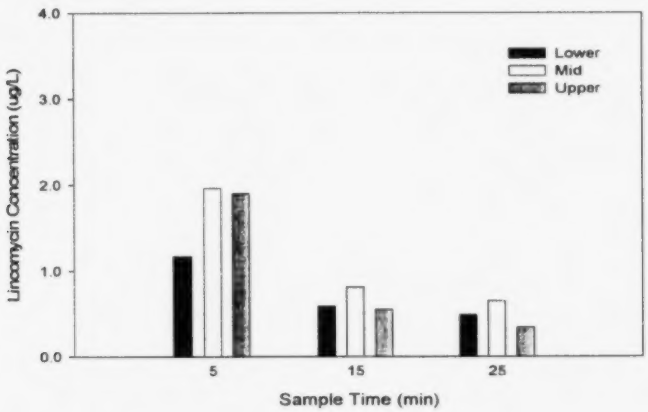
***Results**: PSCI study site 2* - Due to a relatively wet preceding spring and summer, simulated rainfall was easily achieved in the fall of 2004 at PSCI study site 2. Lincomycin and spectinomycin were not detected in any simulated rainfall runoff samples from the control field. Spectinomycin was not detected in any simulated rainfall runoff samples collected from the manure-treated field (78,000 L ha^{-1}). Lincomycin was detected in 100% of the samples collected from the manure-treated field, the maximum concentration being 3.83 μg L^{-1} (Table 8). (Note that similar Figures for one depression (D 1) and the second ephemeral wetland (W 2) on PSCI study site 2 are presented in Appendix B.) Similar to the simulated rainfall runoff experiments carried out at PSCI Study Site 1, concentrations of lincomycin in the runoff water tended to decrease with time (Figure 8).

Table 8. Lincomycin concentrations detected in simulated rainfall runoff collected at three slope positions (upper, mid and lower) from the field at PSCI study site 2 treated in fall 2004 with liquid swine manure at an application rate of 78,000 L ha^{-1}.

Slope position	Time (min)	W1 ($\mu g\ L^{-1}$)	W2 ($\mu g\ L^{-1}$)	D1 ($\mu g\ L^{-1}$)	W3 (control) ($\mu g\ L^{-1}$)
Lower	5	1.17	1.71	3.83	nd
	15	0.59	1.20	2.33	nd
	25	0.49	0.80	1.98	nd
Mid	5	1.96	1.49	1.92	nd
	15	0.81	0.20	1.17	nd
	25	0.65	0.09	0.92	nd
Upper	5	1.90	1.53	1.42	nd
	15	0.55	0.99	1.13	nd
	25	0.34	0.48	0.38	nd

nd: not detected

Figure 8. Lincomycin concentrations ($\mu g\ L^{-1}$) detected in simulated rainfall runoff samples collected in fall 2004 at three slope positions (upper, mid and lower) associated with one of the ephemeral wetlands (W1) from the field at PSCI study site 2 which had been treated with liquid swine manure in fall 2004 at 78,000 L ha^{-1}.



Snow Melt Runoff

In the springs of 2004 and 2005, snow melt runoff samples were collected in 1-L amber glass bottles from the control and manure-treated fields at all study sites (PSCI 1 and 2, and RLSF 1 - 4). During snow melt runoff, samples were collected daily from depressions (Photo 11) or ephemeral wetlands (Photo 12) where runoff water collected. At PSCI study site 2, it was possible to collect runoff water as it entered the two ephemeral wetlands. After runoff was complete, samples were collected weekly at all study sites until water in the depressions or wetlands had completely infiltrated or evaporated. Runoff samples were maintained at 4 °C, until extraction.

Photo 11 Photo 12

Results: *PSCI Study Sites*

At all PSCI study sites, lincomycin and spectinomycin were not detected in any snow melt runoff samples from the control fields. As expected, spectinomycin was not detected in any snow melt runoff samples collected from any of the manure-treated watersheds/fields.

PSCI study site - In the spring of 2004, snow melt runoff was collected from one depression per watershed (total of four) on the four manure-treated watersheds (60,000 and 95,000 L ha^{-1}) and the control watershed. Lincomycin and spectinomycin were not detected in any snow melt runoff samples from the control watershed. Lincomycin was detected in 100% of snow melt runoff samples collected from all depressions at both application rates (Table 9). (Note that the lincomycin concentrations detected in all samples from the PSCI study sites are reported in Appendix C).

Table 9. Lincomycin concentrations (μg L^{-1}) detected in snow melt runoff samples collected from depressions and ephemeral wetlands in the spring following manure application at PSCI study sites 1 and 2.

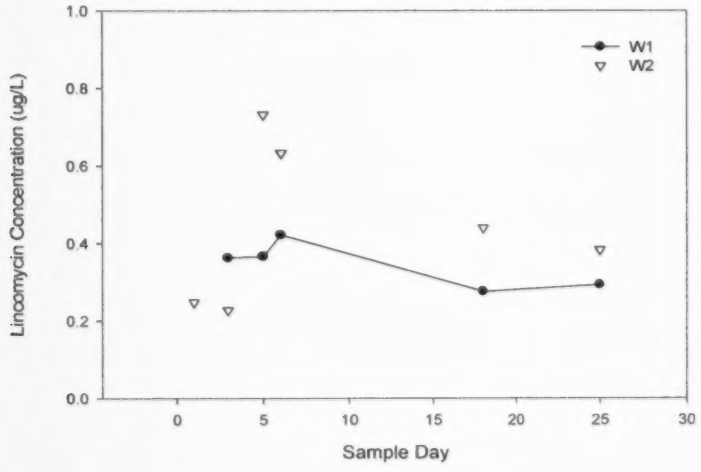
Field cover	Runoff water sample	Application rate ($L\ ha^{-1}$)	Number of samples	Number of depressions*	Number of ephemeral wetlands*	Lincomycin concentration			Frequency of detection (%)
						Mean ($\mu g\ L^{-1}$)	Median ($\mu g\ L^{-1}$)	Range ($\mu g\ L^{-1}$)	
PSCI Study Site 1 - Manure injected in fall 2003									
cereal stubble	depressions	60,000	15	2	0	0.095	0.089	0.038 - 0.181	100
cereal stubble	depressions	95,000	13	2	0	0.313	0.307	0.058 - 0.802	100
PSCI Study Site 2 - Manure injected in fall 2004									
canola stubble	ephemeral wetlands	78,000	11	-	2	0.398	0.366	0.228 - 0.732	100

* samples from manure-treated land only

PSCI study site 2 - In the spring of 2005, snow melt runoff was collected from two ephemeral wetlands on the manure-treated (78,000 L ha^{-1}) quarter section, and one ephemeral wetland and one depression from the control quarter section. Lincomycin and spectinomycin were not detected in any snow melt runoff samples from the control quarter section. Lincomycin was detected in 100% of snow melt runoff samples collected from both ephemeral wetlands on the manure-treated quarter section (Table 9). At this site, it was possible to collect snow melt runoff as it was flowing into the ephemeral wetlands. Concentrations in these samples were higher (0.629 to 1.014 µg L^{-1}) than those detected in samples from the wetlands (Appendix C).

At both PSCI sites, lincomycin concentrations were generally highest three to five days after the commencement of runoff, and then remained relatively constant for the remaining one to two weeks until water infiltrated or evaporated (Figure 9) (Note that similar Figures for all manure-treated watersheds at PSCI study site 1 are presented in Appendix D). Differences in concentrations detected among sites could be due to several factors including: manure application rate, size of the watershed relative to the amount of water collected in the depression or wetland, and the distribution of snow across the manure-treated fields.

Figure 9. Lincomycin concentrations (µg L^{-1}) detected in snow melt runoff samples collected in spring 2005 from two ephemeral wetlands (W1 and W2) at PSCI study site 2 following application of liquid swine manure cropland at 78,000 L ha^{-1} in fall 2004.



Results: *River Lakes Study Sites*

At all RLSF study sites, lincomycin and spectinomycin were not detected in any snow melt runoff samples from the control fields. Spectinomycin was not detected in any snow melt runoff samples collected from any of the manure-treated fields. (Note that the lincomycin concentrations detected in all samples collected from the RLSF study sites are reported in Appendix C. Figures illustrating lincomycin concentrations in snow melt runoff water samples with time for all manure-treated fields at all four RLSF study sites are presented in Appendix D.)

RLSF Study Site 1: Liquid swine manure was applied to this site at an application rate of 79,000 L ha^{-1} and snow melt runoff from fourteen depressions was monitored. Seven depressions were on summer fallow and seven on cereal stubble. Lincomycin was detected in 95% of the samples collected from depressions on the summer fallow portion of this quarter section and 100% of samples from depressions on stubble (Table 10). Lincomycin concentrations ranged from < 0.008 to 4.19 μg L^{-1} and there was no significant difference in lincomycin concentrations detected in runoff from depressions on the summer fallow versus stubble. However, there were 20 samples collected from depressions on the summer fallow, and only 9 from depressions on the crop stubble, suggesting that snow melt runoff was slower to infiltrate on the summer fallow.

RLSF Study Site 2: This site was treated with the liquid component of the swine manure which overflowed into the second storage lagoon. The liquid component was applied using a centre pivot at a rate of approximately 3,000 L ha^{-1}. Snow melt runoff in three ephemeral wetlands and eight depressions was monitored on the quarter section with canola stubble. Lincomycin was detected in 100% of the samples collected from the wetlands and depressions (Table 10). Lincomycin concentrations were lower in the ephemeral wetlands and this may be due to dilution due to snow melt within the wetlands. On the quarter section with cereal stubble, snow melt runoff was monitored from seven depressions and lincomycin was detected in 89% of the samples. Lincomycin concentrations ranged from <0.008 to 2.21 μg L^{-1}. Infiltration was generally rapid on both quarter sections. Only two depressions on each quarter section had more than one sampling and a maximum of five samples was collected from two of the ephemeral wetlands. Lincomycin concentrations detected on this quarter section were similar to those detected on that with the cereal stubble.

Table 10. Lincomycin concentrations (μg L^{-1}) detected in snow melt runoff samples collected from depressions, ephemeral wetlands and dugouts in the spring following manure application at RLSF study sites 1 to 4.

Field cover	Runoff water sample	Application rate (L ha^{-1})	Number of samples	Number Of depressions*	Number of ephemeral wetlands*	Number of dugouts*	Lincomycin concentration			Frequency of detection (%)
							Mean (μg L^{-1})	Median (μg L^{-1})	Range (μg L^{-1})	
RLSF Study Site 1 - Manure injected in fall 2003										
summer fallow	depressions	79,000	20	7	-	-	1.199	1.034	<0.008 - 3.205	95
cereal stubble	depressions	79,000	9	7	-	-	1.727	1.392	0.024 - 4.192	100
RLSF Study Site 2 - Centre pivot manure application in fall 2003										
canola stubble	depressions	3,000	10	8	-	-	0.805	0.498	0.131 - 2.214	100
canola stubble	ephemeral wetlands	3,000	13	-	3	-	0.457	0.385	0.155 - 0.825	100
cereal stubble	depressions	3,000	9	7	-	-	0.935	1.027	<0.008 - 1.396	89
RLSF Study Site 3 - Manure injected in spring 2004										
canola stubble	depressions	90,000	15	7	-	-	0.020	0.008	0.004 - 0.148	100
canola stubble	ephemeral wetlands	90,000	20	-	4	-	0.012	0.005	<0.008 - 0.112	85
RLSF Study Site 4 - Manure injected in fall 2004										
pasture land	depressions	88,000	25	10	-	-	0.263	0.211	0.005 - 0.594	100
pasture land	ephemeral wetlands	88,000	16	-	4	-	0.161	0.130	0.071 - 0.393	100
pasture land	dugouts	88,000	5	-	-	1	0.115	0.085	0.044 - 0.201	100
pasture land	depressions	110,000	32	12	-	-	0.385	0.395	0.036 - 0.844	100
pasture land	dugouts	110,000	6	-	-	1	0.210	0.213	0.163 - 0.276	100

*samples from manure applied land only

RLSF Study Site 3: This site consisted of two quarter sections with canola stubble, which received manure in May 2004 at a rate of approximately 90,000 L ha^{-1}. Snow melt runoff was monitored from seven depressions and four ephemeral wetlands on these quarter sections. Lincomycin was detected in 100 and 85% of these samples, respectively (Table 10). Lincomycin concentrations, which ranged from < 0.008 to 0.148 µg L^{-1}, were lower than those detected at other study sites that had similar manure application rates. Similarly, the mean lincomycin concentration of 0.020 µg L^{-1} was much lower than those (0.095 and 0.313 µg L^{-1}) from PSCI study sites 1 and 2 (Table 9) and those from RLSF study sites 1 (1.20 and 1.73 µg L^{-1}) and 4 (0.115 to 0.385 µg L^{-1}) (Table 10). This was expected because runoff samples from the other study sites were collected approximately five months after application following winter conditions, whereas the samples from RLSF study site 3 were collected approximately ten months after manure application, and this period included the summer months when soil temperatures were the highest and the study site was being farmed.

RLSF Study Site 4: This site consisted of three quarter sections of pasture land, one served as a control and the other two were treated with manure. Snow melt runoff water in ten depressions and four ephemeral wetlands was monitored on the quarter section which received manure at a rate of 88,000 L ha^{-1} and lincomycin was detected in all samples (Table 10). Twelve depressions were monitored in the quarter section treated at 110,000 L ha^{-1} and lincomycin was detected in 100% of the samples collected. Lincomycin concentrations ranged from 0.005 to 0.844 µg L^{-1}.

A dugout was situated on each quarter section at this site and water from each dugout was also monitored. Lincomycin was detected in 100% of the samples collected from the two manure-treated quarter sections and concentrations ranged from 0.044 to 0.276 µg L^{-1} (Table 10). Mean lincomycin concentrations were 0.115 and 0.210 µg L^{-1} for the 88,000 and 110,000 L ha^{-1} rates of manure application, respectively. For both depressions and dugouts, lincomycin concentrations were higher for the quarter section of pasture land that received the higher manure application rate (110,000 L ha^{-1}). However, when compared to fall applications of manure to RLSF cropland site 1, lincomycin concentrations in runoff water in depressions were lowest from the pasture land study site, even though it received the highest rate of manure application.

Objective 5 - *To assess the potential for leaching/preferential flow of these two antimicrobials to shallow ground water by monitoring shallow ground water under manure treated fields.*

Ground Water Study

At PSCI Study Site 1, ground water samples (1-L) were collected from the single piezometer installed in each defined watershed (Photo 13). In the fall of 2003, ground water samples were collected both before and after manure application and then, following spring snow melt runoff, monthly until mid-October 2004. Ground water samples were similarly collected in 2005.

Photo 13

Results: The fall of 2003 was very dry and, as a consequence, ground water was present in only two of the five piezometers, one being in the control watershed and the other in one of the watersheds treated with liquid swine manure at 60,000 L ha^{-1}. Ground water samples were collected from all five piezometers during 2004 and 2005, but not on a consistent basis. In the first year after manure application (fall 2003 through fall 2004), lincomycin and spectinomycin were not detected in any ground water samples from the control watershed. Spectinomycin was not detected in any ground water samples from the manure-treated watersheds in any year.

In the first year after manure application (fall 2003 to fall 2004), lincomycin was detected in ground water samples from two manure-treated watersheds, one at the higher manure application rate (95,000 L ha^{-1}) and the other at the lower rate (60,000 L ha^{-1}) (Table 11). Generally, lincomycin concentrations were higher in samples collected from the watersheds which received the higher manure application rate. The frequency of lincomycin detection was also higher on the watershed receiving the higher manure application rate (71% versus 25%) (Table 11). Due to dry conditions, only a few ground water samples were collected from the other two manure-treated watersheds [60,000 L ha^{-1} (3 samples), 95,000 L ha^{-1} (2 samples)], and lincomycin was not detected in any of these five samples.

In the second year after manure application (fall 2004 to fall 2005), lincomycin was detected in all samples from all manure-treated sites, with concentrations ranging from < 0.001 to 0.003 μg L^{-1} (Table 11). Mean, median and maximum lincomycin concentrations were similar for low and high manure application rates.

Lincomycin was also detected in ground water samples collected from the control watershed. The frequency of detection was 66%, and the mean, median and maximum concentrations detected were 0.001, < 0.001 and 0.002 μg L^{-1}, respectively (Table 11). These concentrations may have been due to the previous liquid swine manure application in the fall of 2002.

Table 11. Lincomycin concentrations (μg L^{-1}) detected in ground water from PSCI study site 1 over two years

Sampling Years	Application rate in fall 2003 (L ha^{-1})	Number of samples	Number of producing piezometers*	Lincomycin concentration			Frequency of detection (%)
				Mean (μg L^{-1})	Median (μg L^{-1})	Maximum (μg L^{-1})	
PSCI Study Site 1							
Fall 2003	60,000	1	1	nd	nd	nd	0
	95,000	0	-	-	-	-	-
	control	1	1	nd	nd	nd	0
April to October 2004	60,000	11	2	< 0.001	nd	0.001	25
	95,000	7	2	0.026	0.004	0.154	71
	control	8	1	nd	nd	nd	0
April to October 2005	60,000	14	2	0.001	< 0.001	0.003	78
	95,000	16	2	0.001	0.001	0.003	81
	control	9	1	< 0.001	< 0.001	0.002	66

nd: not detected

*Due to dry conditions in the fall of 200, no ground water was present in 3 piezometers. Single water samples were collected only from the control watershed and from one manure-treated watershed (60,000 L ha^{-1})

Discussion of Results

Objective 1 - *To develop analytical methods to detect residues of lincomycin and spectinomycin in liquid swine manure, manure-treated soil, and in surface and ground waters.*

Analytical methods were first developed for the analysis of lincomycin and spectinomycin in surface and ground waters. Pressurized liquid extraction methods for extraction of the solids component of liquid swine manure and manure-treated soil using water were then developed. Following dilution of the resulting aqueous extracts from these solid matrices, the diluted extracts were extracted and analyzed according to the water methods. The liquid component of the liquid swine manure was also diluted and similarly analyzed.

Surface and Ground Waters

Lincomycin was efficiently extracted from water using a solid-phase extraction cartridge which utilized a co-polymer sorbent designed to have a hydrophilic-lipophilic balance (HLB) whereas spectinomycin was similarly extracted using a weak cation exchange (WCX) resin. Since spectinomycin was not retained by the HLB cartridge, a HLB cartridge was stacked on top of the WCX cartridge to effect cleanup of the water extract in order to minimize suppression of spectinomycin ionization during LC-MS-MS analysis.

Hydrophilic interaction chromatography (HILIC) provided baseline separation of the two antimicrobials and, when interfaced to atmospheric pressure chemical ionization tandem mass spectral analysis, sensitivity was 0.008 and 0.2 $\mu g\ L^{-1}$ for lincomycin and spectinomycin, respectively, based on a 100-mL water sample. Method sensitivity could be increased by using a larger water sample (for example, by a factor of 5 for a 500-mL water sample). Recoveries from river water fortified at 1 and 100 $\mu g\ L^{-1}$ ($n = 9$) were $103 \pm 10\%$ and $108 \pm 7\%$ for lincomycin and spectinomycin, respectively.

Liquid Swine Manure and Manure-Treated Soil

Liquid swine manure liquid component: The liquid component (2 mL) of the swine manure was diluted 100-fold with deionized water and extracted by the solid-phase extraction procedures used for the water samples. LC-MS-MS analysis of the resulting extracts resulted in limits of quantification of 0.040 and 6.0μg L^{-1} for lincomycin and spectinomycin, respectively. Corresponding recoveries were 78 ± 7% and 84 ± 2% from control samples fortified at 1 and 100 μg L^{-1} (n = 9). This level of sensitivity was more than adequate for the antimicrobial excretion study and the manure storage study.

Liquid swine manure solids component: The solids component (1 g) of the liquid hog manure was subjected to pressurized liquid extraction and the resulting extract diluted to 200 mL with deionized water. The diluted extract was then extracted by the solid-phase procedures used for the water samples. LC-MS-MS analysis of the resulting extract resulted in limits of quantification of 0.040 and 6.0 μg L^{-1} for lincomycin and spectinomycin, respectively. Reliable recoveries were obtained for both antibiotics at the 50 and 100 μg kg^{-1} fortification levels and were 70 ± 10% and 62 ± 12% (n = 9) for lincomycin and spectinomycin, respectively. This level of sensitivity was more than adequate for the antimicrobial excretion study and the manure storage study.

Manure-treated soil: Soil (2 g) was subjected to pressurized liquid extraction and the resulting extract diluted to 200 mL with deionized water. The diluted extract was then extracted by the solid-phase procedures used for the water samples. LC-MS-MS analysis of the resulting extract resulted in limits of quantification of 0.040 and 6.0 μg L^{-1} for lincomycin and spectinomycin, respectively. Reliable recoveries were obtained for both antibiotics at the 1 and 100 μg kg^{-1} fortification levels and were 74 ± 8% and 66 ± 15% (n = 9) for lincomycin and spectinomycin, respectively. This level of sensitivity was adequate to monitor the dissipation of the antimicrobials in manure-treated soil.

Objective 2 - *To quantify, by analysis of liquid swine manure, the extent of excretion of these antimicrobials used to prevent post-weaning diarrhoea in swine.*

Antimicrobial Excretion

Based on the amount of lincomycin and spectinomycin administered to the weanling pigs and the volume of liquid manure produced during the excretion study carried out at the PSCI barn, maximum antimicrobial concentrations expected in the total volume of nursery manure at the end of the excretion study would have been 9,535 μg L^{-1} of lincomycin and 8,035 μg L^{-1} of spectinomycin, assuming 100% excretion of both antimicrobials and no degradation in the manure. Detected concentrations in the nursery manure (liquid plus solids components) at the end of the excretion study (day 42) were 1,990 and 135 μg L^{-1} (Table 2). These concentrations correspond to approximately 20.9% of the lincomycin and 2.4% of the spectinomycin administered to the pigs during the excretion study. The proportions of the administered antimicrobials actually excreted may have been greater since there may have been degradation of both antimicrobials in the nursery manure below the study room during the 42-day study. If degradation occurred, the observed proportions would be an underestimation of the amounts excreted. Winckler and Grafe (2001) reported higher excretion rates for tetracycline from swine after seven days and noted that the percent excreted was dependent on the dose administered. The proportions excreted were 42 and 72% following administration of tetracycline at 1,000 and 2,500 mg kg^{-1} in their feed for five days.

Assuming both antimicrobials were excreted to the same extent in the nursery manure and that no degradation of either antimicrobial occurred in the manure, the ratio of lincomycin to spectinomycin expected in the nursery manure of the PSCI barn would be that of the relative amounts of the antimicrobials in the L-S 20 Premix; that is, 1.19:1. However, the ratio of the concentrations of lincomycin to spectinomycin in the nursery manure at the end of the excretion study was approximately 14.7:1 (from data in Table 2). The approximate 12-fold increase in this ratio indicates either that a lower proportion of the administered dose of spectinomycin was excreted by the weanling pigs and/or that spectinomycin was less stable in the nursery manure.

This increase in the lincomycin to spectinomycin ratio may also be indicative of the relative amounts of the two antimicrobials in the liquid manure applied to crop and pasture land. Thus, at the PSCI barn where essentially equal amounts of lincomycin and spectinomycin were administered to the pigs, there would be about 12 times as much lincomycin as spectinomycin in the manure applied to crop land. In contrast, at the RLSF barn where approximately 24 times more lincomycin was used than spectinomycin, there would be approximately 290 times more lincomycin in the applied manure, assuming a similar 12-fold increase in the lincomycin to spectinomycin ratio in the manure due to excretion/degradation. However, the relative amounts of these antimicrobials in the liquid manure applied to crop and pasture land would also be dependent on their relative stability in the manure during lagoon storage. For example, if spectinomycin was preferentially degraded to a greater extent than lincomycin during lagoon storage, then the lincomycin to spectinomycin ratios would be even larger.

Objective 3 - *To determine the extent to which both antimicrobials persist in the manure during simulated storage and in soil following manure injection into cropland or pasture land.*

Persistence during Simulated Manure Storage

Because the PSCI lagoon was enclosed with a plastic cover, the liquid manure in the lagoon was most likely anaerobic. Consequently, during the simulated storage study, the persistence of lincomycin and spectinomycin in fortified manure was monitored in the laboratory in covered containers. The average temperature (approximately 20°C) of the manure in the laboratory study would have been higher than that in earthen lagoon storage from spring to early fall, and considerably higher than that for winter lagoon storage from early fall to spring. Thus, the laboratory conditions most likely represent a worst-case scenario for antimicrobial degradation.

There were no reports in the literature with respect to persistence of either lincomycin or spectinomycin in liquid swine manure when this study was initiated. A half-life of 105 days for tetracycline in swine manure had been reported by Winckler and Grafe (2001), whereas much shorter

half-lives were measured by Kuhne et al. (2000) in aerated (4.5 days) and non-aerated (9 days) liquid swine manure. More recently, Schlusener et al. (2006) have reported half-lives of 41, 130, 6 and > 180 days for erythromycin, roxithyromycin, salinomycin and tiamulin, respectively, in liquid swine manure.

Lincomycin: The dissipation of lincomycin in liquid manure showed a biphasic pattern. Lincomycin concentrations decreased more rapidly from the target concentration (40 μg L^{-1}) during the first three days of the study and then, from day 6, remained relatively constant (mean concentrations were 31.8 ± 61 and 35.3 ± 4.0 μg L^{-1}) or decreased very slowly over the next five months (Tables 3a and 3b; Figures 3a and 3b). This decrease corresponds to approximately 12 to 20% of the amount of lincomycin added to the liquid manure. Thus, even at room temperature, there was relatively little dissipation of lincomycin during simulated storage under laboratory conditions. These results indicate that lincomycin would persist in the manure during six months of lagoon storage and be present in the manure when injected into crop or pasture land as a plant nutrient source. Lincomycin was detected in all samples of liquid manure applied to the PSCI and RLSF study sites (Table 5), confirming the prediction of the manure storage study.

Spectinomycin: In contrast to lincomycin, concentrations of spectinomycin decreased substantially from the target concentration (400 μg L^{-1}) during the first 6 days of the study and then stabilized at approximately 65 μg L^{-1} for the remainder of the study (Tables 4a and 4b; Figures 5a and 5b). This decrease corresponds to approximately 84% of the amount of spectinomycin added to the liquid manure. This decrease in spectinomycin concentration was not as evident in liquid manure (liquid plus solids components) fortification experiments, most likely because the fortified manure was maintained at -40°C prior to extraction (for up to two months). The mean recovery of spectinomycin from fortified liquid manure (liquid plus solids components) was 58 ± 16%.

Lincomycin and Spectinomycin Concentrations in Liquid Manure Applied to Study Sites

The actual concentrations of the antimicrobials in the liquid manure from the PSCI barn applied to crop land would depend on the extent of dilution of the nursery manure with that from animals not

administered the antimicrobials. Based on the estimated total volume of liquid swine manure applied from the PSCI lagoon in the fall of 2003 and the estimated volume of nursery manure produced in the PSCI barn in the preceding12-month period, antimicrobial concentrations in the nursery manure would have been diluted by a factor of approximately 13; that is, from 1,990 and 135 μg L^{-1} to 153 and 10 μg L^{-1} for lincomycin and spectinomycin, respectively. The mean concentration of lincomycin in the manure from the PSCI lagoon applied to crop land in fall 2003 was 132 μg L^{-1}, whereas that for fall 2004 was 122 μg L^{-1} (Table 5). Lincomycin concentrations in the applied manure at both sites ranged from 45.8 to 231 μg L^{-1}. These concentrations were within the range (2.5 to 240 μg L^{-1}) Campagnola et al. (2002) reported for concentrations of lincomycin in eight swine lagoons in Iowa and Ohio.

Based on the mean concentrations of lincomycin in the liquid manure applied at PSCI study sites 1 and 2, the expected mean concentrations of spectinomycin would have been approximately 9 and 8 μg L^{-1}, respectively; that is, very close to the limit of quantification of the analytical method (6 μg L^{-1}). Even lower spectinomycin concentrations would be expected in the liquid manure from the RLSF lagoons because of the much lower use of spectinomycin. Since the simulated storage study indicated that spectinomycin may be less stable than lincomycin in liquid manure during lagoon storage (see following discussion), it is not surprising that spectinomycin was not detected in the manure applied to crop and pasture land at the PSCI and RLSF study sites.

Persistence in Soil

There were no reports on the persistence in soil of either lincomycin or spectinomycin in the literature when this study was initiated. Schlusener et al. (2003) reported that tiamulin was still present in soil (0.7 μg kg^{-1}) 275 days after being treated with liquid swine manure containing 43 μg kg^{-1} tiamulin and 11 μg kg^{-1} salinomycin. No salinomycin was detected was detected in the soil. More recently, Aga et al. (2005) reported a half-life of 21 days for oxytetracycline in soil. However, the total tetracycline concentration was shown to be relatively constant for up to 150 days when an ELISA analysis was used.

Lincomycin: Immediately after liquid manure application, lincomycin was detected in the upper 5 cm of manure-treated soil from all three slope positions (upper, mid and lower) at both PSCI study sites in concentrations ranging from 200 to 1,595 $\mu g\ kg^{-1}$ (Tables 6a and 6b). At study site 1 where the soil was very dry when the manure was injected, placement of lincomycin in the upper 5 cm of soil was not consistent across slope positions (Table 6a). This may reflect the fact that the liquid manure was not injected by the applicator to a consistent depth because of the very dry soil. Following snow melt runoff the following spring, lincomycin was detected in the soil until the end of June, and mainly at the upper slope position. Dissipation of lincomycin from this upper layer of soil was probably by microbial degradation.

In contrast, at study site 2 where the soil was much wetter when injected with liquid manure, concentrations of lincomycin varied by slope position and were generally of the order lower slope > mid slope > upper slope (Table 6b). This may reflect the fact that the liquid manure was injected by the applicator to a greater depth at the mid and upper slope positions so that less lincomycin was placed in the upper 5 cm of soil. At this site following snow melt runoff, lincomycin was detected only at the lower slope positions. This may be due, in part, to lincomycin moving down the slope with snow melt runoff and accumulating at the lower slope position. Lincomycin persisted in the soil at this site until mid July.

Sufficient lincomycin was placed in the runoff-soil interaction zone during manure injection such that it was detected in simulated rainfall runoff samples (Tables 7 and 8). It would be expected, then, that until degraded in the soil, lincomycin placed in the runoff-soil interaction zone would be available for transport in rainfall or snow melt runoff into surface waters, such as farm dugouts or wetlands. In the springs following manure application at both PSCI study sites, lincomycin was detected in snow melt runoff in 100% of the runoff water samples collected from depressions and ephemeral wetlands (Table 9). Similarly, at the four RLSF study sites, lincomycin was detected in essentially all of the snow melt runoff samples (Table 10) from depressions, ephemeral wetlands and dugouts.

Any lincomycin placed below the runoff-soil interaction zone during manure injection, or subsequently leached below this zone, would be available for leaching to ground water. Lincomycin was not detected in ground water samples in the fall of 2003, but was detected in samples collected in 2004 and 2004 (Table 11).

Spectinomycin: Although there was a substantial decrease (approximately 84%) in spectinomycin concentration during the simulated liquid manure storage study, spectinomycin would also persist in the manure during six months of lagoon storage and be present in the manure when injected into crop or pasture land. However, spectinomycin was not detected when samples of the liquid manure applied to the PSCI and RLSF study sites were analyzed, indicating that lagoon storage decreased spectinomycin concentrations to less than the limit of quantification (6 $\mu g\ L^{-1}$) of the analytical method. Following liquid manure injection, spectinomycin was not detected in the upper 5 cm of soil, indicating that concentrations in soil were also < 6 $\mu g\ kg^{-1}$. Thus, although spectinomycin may have been placed in or below the runoff-soil interaction zone during manure injection, concentrations in the soil were such that it was not detected in samples of simulated rainfall runoff, snow melt runoff or ground water.

Objective 4 - *To assess the potential for their transport in surface runoff from manure-treated land into surface water bodies using simulated rainfall runoff experiments and by monitoring snow melt runoff.*

There were no reports in the literature of transport of either lincomycin or spectinomycin in rainfall or snow melt runoff when this study was initiated. However, lincomycin had been detected in surface waters. Calamari et al. (2003) had sampled the rivers Po (7 sites) and Lambro (1 site) in Italy and lincomycin was detected in all samples at concentrations ranging from 3.13 to 249 $\mu g\ L^{-1}$. A similar survey of 139 streams in 30 states in the USA by Kolpin et al. (2002) resulted in a 19% detection frequency (104 sites) of lincomycin with a median concentration of 0.06 $\mu g\ L^{-1}$. Campagnolo et al. (2001) sampled rivers adjacent to large-scale confined swine and poultry feeding

operations in the USA and lincomycin was detected in one river. In a very recent study in Alberta, Canada in which 22 streams were sampled for a variety of pharmaceuticals including veterinary antimicrobials, lincomycin was detected in one of the streams (Forrest et al. (2006).

Simulated Rainfall Runoff

In fall 2003, spring 2004 and fall 2004 simulated rainfall runoff experiments, lincomycin concentrations were generally highest in the 5-minute runoff sample and then progressively decreased in the 15- and 25-minute samples (Tables 7 and 8, Figure 7, Appendix B). This pattern was expected because the lincomycin detected in the simulated rainfall runoff samples would have originated in the runoff-soil interaction zone, which is considered to be the upper 1 cm of soil. Concentrations in the runoff water decreased with time because there would be decreasing amounts of lincomycin available in the runoff-soil interaction zone to interact with the runoff water as runoff proceeded. The amount of lincomycin in the runoff-soil interaction zone would have decreased with time due to lincomycin removal in runoff as well as leaching of lincomycin with the infiltrating water below the runoff-soil interaction zone.

After snow melt runoff in spring 2004, lincomycin concentrations in simulated rainfall runoff water were lower in the spring experiments than those carried out the previous fall on the same watershed (WS1) at all slope positions (Table 7). The presence of lincomycin in the runoff water during the spring experiments indicates that lincomycin was relatively stable in the runoff-soil interaction zone during the winter months (November to March). The reduced concentrations in the spring runoff experiments most likely indicate some degradation of lincomycin within the runoff-soil interaction zone and/or leaching out of this zone.

In the spring 2004 experiments, it was anticipated that runoff samples collected from the lower slope positions would have higher lincomycin concentrations than those from the mid and upper slope positions because of down slope movement of chemical during snow melt runoff. This pattern was evident in two (WS3 and WS4) of the four watersheds (Table 7) but there was no significant difference amongst the slope positions (p = 0.47, 0.50 and 0.44 for the 5-, 15- and 25-minute

samples, respectively). The lack of a consistent pattern across slope positions may have resulted, in part, because the depth of manure injection by the applicator may not have been consistent due to the very dry soil conditions. Variation in injection depth would affect the amount of lincomycin placed in the runoff-soil interaction zone.

The lincomycin concentrations detected in runoff samples collected in fall 2003 (Table 7) were lower than those in fall 2004 (Table 8) and may reflect, in part, the lower application rate applied in fall 2003 (60,000 versus 78,000 L ha^{-1}). However, the difference may also be due the dry soil conditions in the fall of 2003. Simulated rainfall was difficult to achieve because the water preferentially infiltrated the soil: thus, lincomycin may have leached below the runoff-soil interaction zone with the infiltrating water.

Since simulated rainfall runoff experiments were carried out within a week of manure application each fall, it was expected that there would be no significant difference in lincomycin concentration in runoff water samples from the three slope positions (upper, mid and lower) at each of the three sampling times (5, 15, 25 min). For the fall 2004 experiments (Table 8), no significant difference in lincomycin concentrations was observed (p = 0.67, 0.45 and 0.30 for the 5-, 15- and 25-minute samples, respectively). Because of limited data for fall 2003 (Table 7), a similar assessment could not be made.

Snow Melt Runoff

Lincomycin concentrations in snow melt runoff samples from the PSCI study sites 1 and 2 (Table 9) were of the same order of magnitude as those in corresponding simulated rainfall runoff samples (Tables 7 and 8) indicating that there was little dissipation of lincomycin from the runoff-soil interaction zone over the winter months, most likely because of cold soil temperatures. At a given study site, concentrations of lincomycin in snow melt runoff water which collected in depressions were dependent upon the application rate of liquid manure. For example, at PSCI study site 1, the mean concentration of lincomycin detected in samples collected from depressions on watersheds (cereal stubble) treated with liquid manure at 95,000 L ha^{-1} in fall 2003 was higher (0.313 µg L^{-1})

than that (0.095 μg L^{-1}) from watersheds treated at 60,000 L ha^{-1} (Table 9) Similarly, at RLSF study site 4, mean concentrations in runoff in depressions were 0.263 and 0.385 μg L^{-1} for liquid manure application rates of 88,000 and 110,000 L ha^{-1}, respectively (Table 10). It should be noted that all depressions from PSCI and RLSF study sites were directly treated with liquid manure (either by injection or centre pivot) which may have contributed, in part, to the lincomycin concentrations detected in runoff water from depressions. Runoff water in depressions in manure-injected fields (RLSF study sites 1 and 4) had higher lincomycin concentrations than that in ephemeral wetlands, most likely because the ephemeral wetlands were not directly injected with liquid manure.

At PSCI study site 2, runoff water was collected as it entered the two ephemeral wetlands (Appendix C). The detection of lincomycin in these three samples (0.629 to 1.014 μg L^{-1}) directly confirms the transport of lincomycin in snow melt runoff. Lincomycin concentrations in these samples were higher than those detected in samples of wetland water (Table 9), most likely due to dilution of the wetland water by snow melt from snow which had accumulated within the ephemeral wetland.

At RLSF study site 1, the lincomycin concentrations detected in snow melt runoff water in the depressions on crop stubble were higher than those detected in depressions on the summer fallow (Table 9). Because the soil was so dry, this difference may be due to the manure not being injected as deeply into crop stubble as it was on summer fallow.

The lowest concentrations of lincomycin in snow melt runoff water in depressions and ephemeral wetlands were detected on RLSF study site 3, the site that was injected with manure in spring 2004. Lower concentrations at this study site were expected because of the longer time between manure application and the collection of the snow melt runoff water. Runoff water samples from the PSCI and other RLSF study sites were collected after approximately five months of winter conditions, whereas the samples from RLSF study site 3 were collected approximately ten months after manure application, and this period included the summer months when soil temperatures were the highest and the study site was being farmed.

The highest concentrations of lincomycin were detected in snow melt runoff water in depressions on RLSF study sites 1 and 2 at which manure was applied by injection and centre pivot, respectively. Since the lowest injected application rate (78,000 L ha^{-1}) was applied at RLSF study site 1 (Table 10), the high lincomycin concentrations in the runoff water may indicate a much shallower injection depth such that more lincomycin was placed in the runoff-soil interaction zone. They may also be due to less dilution of the runoff because of lower snow accumulation in the winter of 2003-2004 than in 2004-2005. At RLSF study site 2, the liquid component of the liquid manure was applied directly to the soil surface using the centre pivot. Although applied at a very low application rate (3,000 L ha^{-1}), essentially all of this application would have remained within the runoff-soil interaction zone. Thus, the amount of lincomycin placed in the runoff interaction zone was similar to that resulting from injection to a 15-cm depth at RLSF study site 1 for which a relatively much smaller proportion of the applied manure would have been placed in the runoff-soil interaction zone.

Lincomycin concentrations detected in the dugouts located on RLSF study site 4 were somewhat lower than those in ephemeral wetlands or depressions on fields with the same manure application rates (Table 10). The lower concentrations were most likely because of dilution by water in the dugout and by snow melt from snow trapped in the dugout. The lower mean lincomycin concentration detected in the ephemeral wetlands (0.161 µg L^{-1}) compared to depressions (0.263 µg L^{-1}) can also be attributed to dilution from snow contained within the ephemeral wetland.

Objective 5 - ***To assess the potential for leaching/preferential flow of these two antimicrobials to shallow ground water by monitoring shallow ground water under manure treated fields.***

There were no reports in the literature of detections of spectinomycin in ground water and only one report of lincomycin in ground water when this study was initiated. Campagnolo et al. (2001) had sampled wells adjacent to large-scale confined swine and poultry feeding operations in the USA and lincomycin was detected in one well.

Ground Water Study

The four watersheds at PSCI study site 1 that were treated with manure in fall 2003 had been previously treated with manure two years previously (fall 2001). The control watershed was previously treated with manure in fall 2002. Thus, it is possible that a portion of the lincomycin concentrations detected in ground water may have been due to past fall manure applications.

Because of dry conditions, ground water samples were collected only from the control and one manure treated watershed (60,000 L ha^{-1}) in fall 2003. Neither antimicrobial was detected in these samples (Table 11), which were collected two years after the previous manure application.

Spring and summer 2004 were much wetter and ground water samples were collected from all four manure-treated watersheds. Lincomycin was detected in ground water from one watershed treated at the higher manure application rate (95,000 L ha^{-1}) and the other at the lower rate (60,000 L ha^{-1}) (Table 11). Lincomycin was more frequently detected (71 versus 25%), and mean concentrations (0.026 versus < 0.001 µg L^{-1}) were higher in samples from the watershed which received the higher application rate.

Spring and summer 2005 were also wetter than 2003 and lincomycin was detected at a higher frequency in ground water samples from all four manure-treated watersheds than the previous year (Table 11). However, lincomycin concentrations in the ground water were much lower. In addition, lincomycin was also detected in ground water samples collected from the control watershed. The frequency of detection was 66%, and the mean, median and maximum concentrations were of the same order of magnitude as those from the manure-treated watersheds. The detection of lincomycin in ground water from the control watershed may be due to the liquid swine manure application made in fall 2002; that is, three years previously. Since the treated watersheds had also been previously treated with liquid swine manure, it is not clear whether the lincomycin detected in the ground water samples originated from the previous applications or the application made in fall 2003. However, the detection of lincomycin in the ground water confirms that when present in liquid manure applied to cropland as a plant nutrient source, leaching to ground water can occur under prairie conditions.

Conclusions

1. Both lincomycin and spectinomycin were present in the liquid swine manure in amounts equivalent to 20.9 and 2.4%, respectively, of the doses administered in feed after a six-week excretion study.
2. Lincomycin was much more stable in the stored liquid swine manure than spectinomycin.
3. Spectinomycin was not detected in the applied lagoon manure. Consequently, it was not detected in treated soil, in simulated rainfall runoff, in spring snow melt runoff or in ground water.
4. Lincomycin was present in the applied lagoon manure and persisted in the upper 5 cm of soil from fall application until mid-June to mid-July the following year.
5. Immediately after fall manure application, lincomycin was detected in simulated rainfall runoff from all slope positions (upper, mid and lower).
6. During spring snow melt following fall or spring application of liquid swine manure, lincomycin was detected in dugout water and in snow melt runoff water in essentially all depressions and ephemeral wetlands.
7. Lincomycin persisted in the water in depressions and ephemeral wetlands until all of the water had infiltrated/evaporated.
8. Lincomycin was detected more frequently in ground water two years after manure application.

Significance to the Swine Industry

Many of the results of this study are significant to swine production in Saskatchewan. First, the study shows that antimicrobials used to prevent disease or enhance weight gain in confined animal feeding operations can be excreted in the feces/urine such that detectable concentrations are present in the liquid manure. Second, it is also evident that with the management practice of storing liquid manure in earthen lagoons and applying the manure to crop or pasture land once or twice a year, some antimicrobials can be stable in the stored manure such that they are present in the lagoon manure when applied as a plant nutrient source. Third, since the study showed that some antimicrobials may persist in the upper layer of soil for several months after application, the practice of injecting the manure into soil results in a portion of the antimicrobials being available for

transport in surface runoff into surface water bodies (such as wetlands and dugouts) and the remainder available for leaching to ground water. Thus, the consequence of current management practices (antimicrobial use and use of liquid manure as a plant nutrient source) may result in environmental impacts on aquatic ecosystems.

However, the study shows that producers can also make choices to minimize environmental impacts. The study shows that some antimicrobials may be used that do not have environmental impacts because they are not present in the lagoon manure applied to crop and pasture land. The study also shows that spring application (rather than fall application) of liquid swine manure to soil reduced the amount of antimicrobial available for transport in snow melt runoff into surface water bodies the following spring. Producers could choose a deeper injection depth to minimize antimicrobial transport in surface runoff. While this could potentially increase leaching to ground water, microbial degradation in soil could take place before significant leaching occurs.

100-Word Study Summery

Lincomycin and spectinomycin were excreted in liquid manure from swine administered these antimicrobials in two commercial barns. Lincomycin was more stable than spectinomycin in stored liquid manure and was present in manure applied to study sites, whereas spectinomycin was not. Lincomycin was present in simulated rainfall runoff and in snow melt runoff from manure-treated fields. It was detected in ground water more frequently two years after manure application. Thus, current management practices to utilize livestock manure as a plant nutrient source on crop and pasture land will result in the transport of lincomycin into surface waters via runoff, and into ground water via leaching/preferential flow.

Manuscripts

Rough drafts of two more manuscripts have been written. These manuscripts will be completed and submitted for publication early in 2007. One manuscript discusses antimicrobial transport in simulated rainfall and snow melt runoff, persistence in soil and possible leaching to ground water. The other describes the extent of excretion of these antimicrobials by weanling pigs and the persistence of the antimicrobials during simulated manure storage. These manuscripts will form chapters in Kuchta's Master's thesis.

Personnel

Sandra Kuchta, a graduate student (M.Sc. Program) through the Toxicology Centre, University of Saskatchewan, has been paid a stipend with SADF funds (April 1 to December 31, 2006).

Equipment

No equipment has been rented or purchased during the study. Laboratory and field supplies have been purchased including: chromatography columns, solid-phase extraction materials, accelerated solvent extraction materials, analytical standards, solvents, glassware, and extract vials.

Project Developed Materials

Seven posters and one publication (see publications/presentations) outlining the analytical methods developed, transport of lincomycin and spectinomycin in simulated rainfall runoff and spring snow melt runoff, concentrations in ground water, excretion of the antimicrobials from weanling pigs, and their persistence in liquid swine manure.

Acknowledgements

SADF has been/will be identified as a source of funding on all oral and written presentations.

Publications/Presentations

Previously Reported Publications

K.M. Peru, S.L. Kuchta, J.V. Headley, and A.J. Cessna. Development of a hydrophilic interaction chromatography-mass spectrometry assay for spectinomycin and lincomycin in liquid hog manure supernatant and run-off from cropland. Journal of Chromatography A. (2006), 1107: 152-158.

Oral presentations

Previously Reported Oral Presentation

Sandra L. Kuchta, Kerry M. Peru, John V. Headley, and Allan J. Cessna. 2005. Investigation of lincomycin and spectinomycin in liquid hog manure; presented by Sandra Kuchta at the Western Canada Trace Organic Workshop, Winnipeg, MB, May 2-4, 2005.

Sandra L. Kuchta, Kerry M. Peru, Allan J. Cessna, John V. Headley, and Jane A. Elliott. 2004. Analysis by LC/MS/MS of surface and ground waters for traces of the antibiotics lincomycin and spectinomycin; presented by Sandra Kuchta at the Western Canada Trace Organic Workshop, Saskatoon, SK, April 18-21, 2004.

Poster presentations

Jonathan Bailey, Allan J. Cessna, John V. Headley, Marley J. Waiser, Kerry M. Peru and Sandra L. Kuchta. 2006. Investigation of the stability of chlortetracycline in prairie wetlands by LC-MS/MS; presented at the 19th Annual Tandem Mass Spectrometry Workshop, Lake Louise, Alberta, November 29 – December 2, 2006.

Sandra L. Kuchta, Allan J. Cessna, Kerry M. Peru, John V. Headley, Jane A. Elliott. (2006) Environmental fate and persistence of two antibiotics; presented by Sandra Kuchta at the 41st Annual Western Canada Trace Organic Workshop, Vancouver, BC, April 23-27, 2006.

Previously Reported Poster Presentations

Sandra L. Kuchta, Allan J. Cessna, Kerry M. Peru, John V. Headley, and Jane A. Elliott. 2005. Analysis of lincomycin and spectinomycin in ground water and surface runoff from manure treated fields; presented by Sandra Kuchta at the 26th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, Maryland, November 13-17, 2005.

Kerry M. Peru, John V. Headley, Sandra L. Kuchta, and Allan J. Cessna. 2005. Determination of spectinomycin in liquid hog manure by LC/MS/MS using HILIC separation and weak cation exchange sample extraction; presented by Kerry Peru at the 53nd Annual Conference of the American Society of Mass Spectrometry, San Antonio, Texas, June 5-9, 2005.

Sandra L. Kuchta, Kerry M. Peru, John V. Headley, and Allan J. Cessna. 2004. Excretion of the antibiotics lincomycin and spectinomycin in liquid hog manure and their persistence during manure storage; presented by Sandra Kuchta at the 25th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Portland, Oregon, November 14-18, 2004.

Sandra L. Kuchta, Kerry M. Peru, Allan J. Cessna, John V. Headley, and Jane A. Elliott. 2004. Analysis by LC/MS/MS of surface and ground waters for traces of the antibiotics lincomycin and spectinomycin; presented by Sandra Kuchta at the University of Saskatchewan Life Sciences Poster Competition, May 13-14, 2004, and at the Toxicology Centre, University of Saskatchewan General Meeting Poster Competition, May 20, 2004.

Kerry M. Peru, John V. Headley, Sandra L. Kuchta, Allan J. Cessna. 2004. Analysis of spectinomycin and lincomycin by APCI-LC/MS/MS in simulated rainfall runoff from soils treated with animal manure was presented by Kerry Peru at the 52nd Annual Conference of the American Society of Mass Spectrometry, Nashville, Tennessee, May 23-27, 2004.

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Appendix A

Table 1. Lincomycin concentrations (μg L^{-1}) detected in liquid hog manure collected during fall manure applications.

Table 1a. Fall 2003 manure application – PSCI study site 1

Liquid component (μg L^{-1})	Solids component (μg L^{-1})	Total concentration (μg L^{-1})	% from solids
103	1.85	105	1.76
133	2.94	136	2.17
104	4.05	108	3.76
164	14.94	179	8.34
	Mean	132	4.01
	Std Dev	34	3.02

Table 1b. Fall 2004 manure application – PSCI study site 2

Supernatant (μg L^{-1})	Solids component (μg L^{-1})	Total concentration (μg L^{-1})	% from solids
174	32.7	207	15.8
228	2.84	231	1.23
114	11	125	8.79
36.5	18	55	33.1
67	ns	67	na
45.8	ns	45.8	na
	Mean	122	14.7
	Std Dev	80	13.6

Table 1c. Fall 2004 manure application – RLSF study site 4

Supernatant (μg L^{-1})	Solids component (μg L^{-1})	Total concentration (μg L^{-1})	% from solids
18.7	6.43	25.1	25.6
34.8	3.69	38.5	9.58
	Mean	31.8	17.6

ns: no sample available

Appendix B

Figure 1a) Lincomycin concentrations (μg L^{-1}) detected in simulated rainfall runoff samples collected from cropland that was manure-treated in fall 2003 at rates of 60,000 and 95,000 L ha^{-1} at PSCI study site 1. Samples were collected at three different slope positions (upper, mid, lower) in relation to each watershed. Due to dry conditions in the fall of 2003, experiments were only completed at the control site and one manure-treated site (i) WS1.

i) WS1

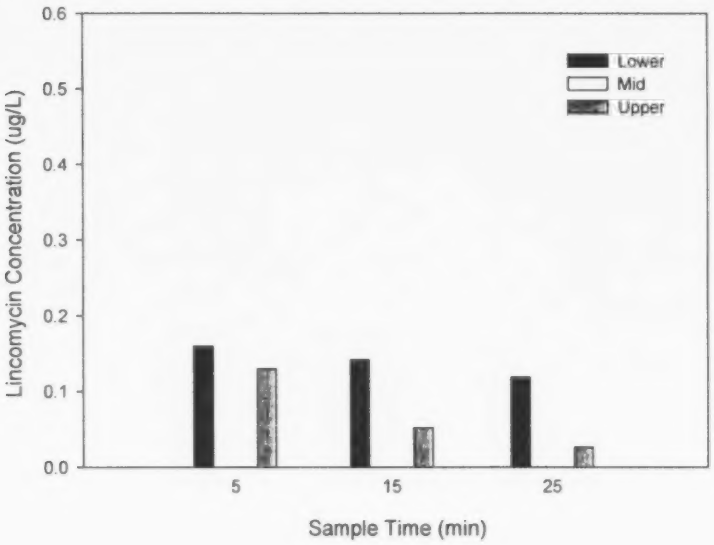
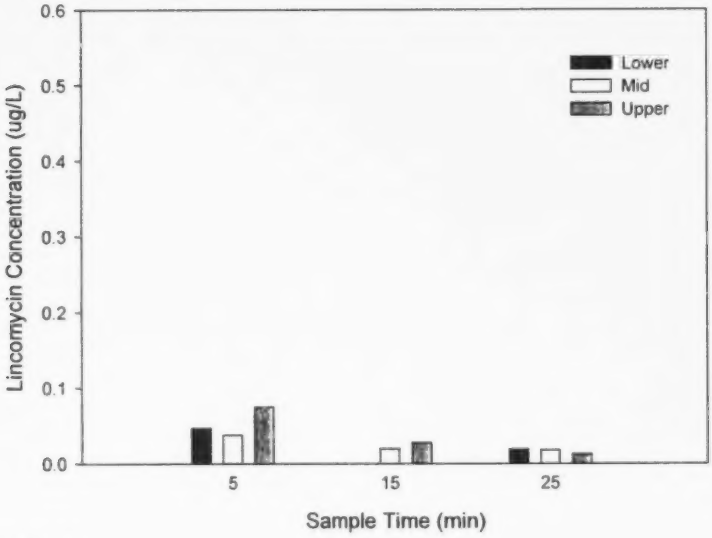
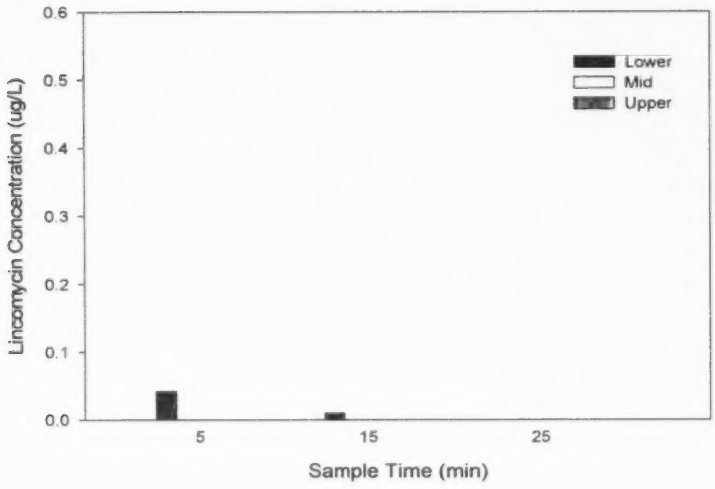


Figure 1b) Lincomycin concentrations (μg L^{-1}) detected in simulated rainfall runoff samples collected from cropland that was manure-treated in fall 2003 at rates of 60,000 and 95,000 L ha^{-1} at PSCI study site 1. Samples were collected at three different slope positions (upper, mid, lower) in relation to each watershed (i) WS1 (ii) WS2 (iii) WS3 and (iv) WS4. These experiments were repeated in May 2004 after snow melt runoff due to dry conditions in the fall of 2003.

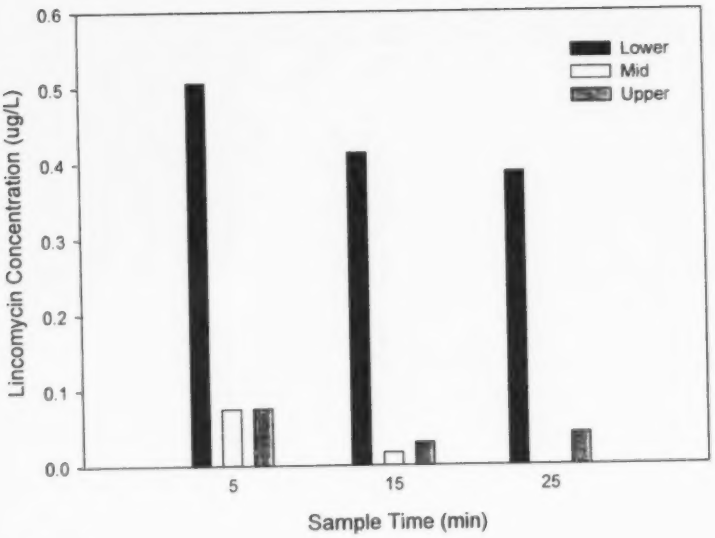
i) WS1



ii) WS2



iii) WS3



iv) WS4

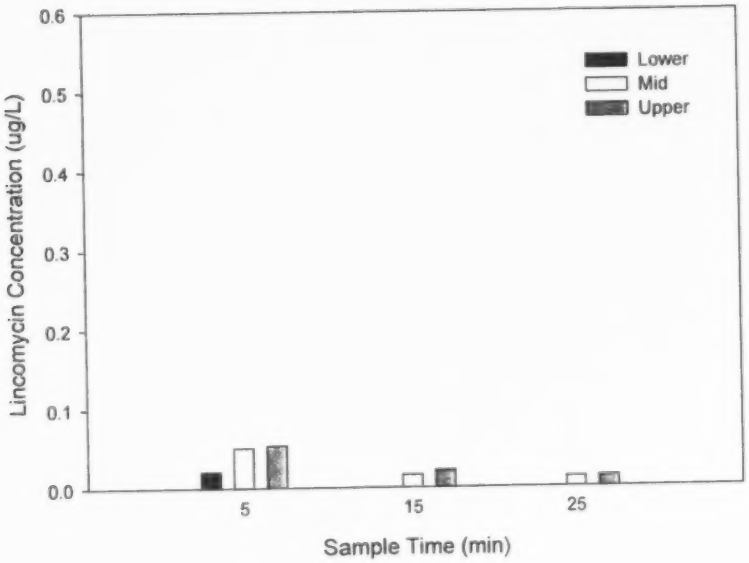
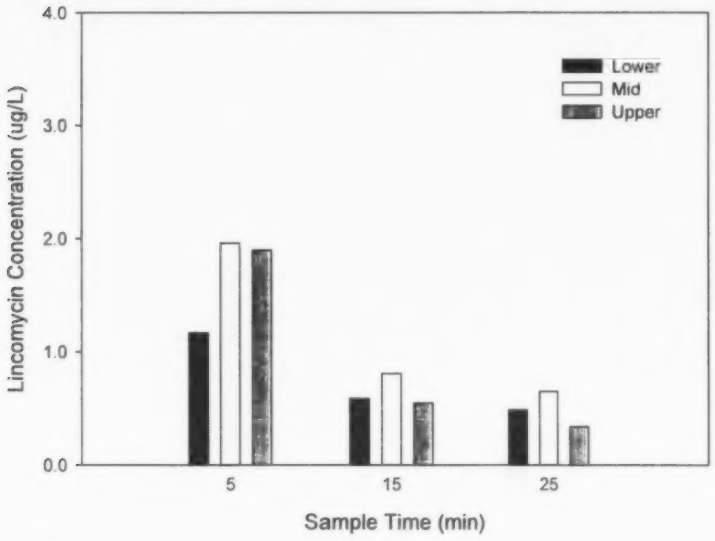
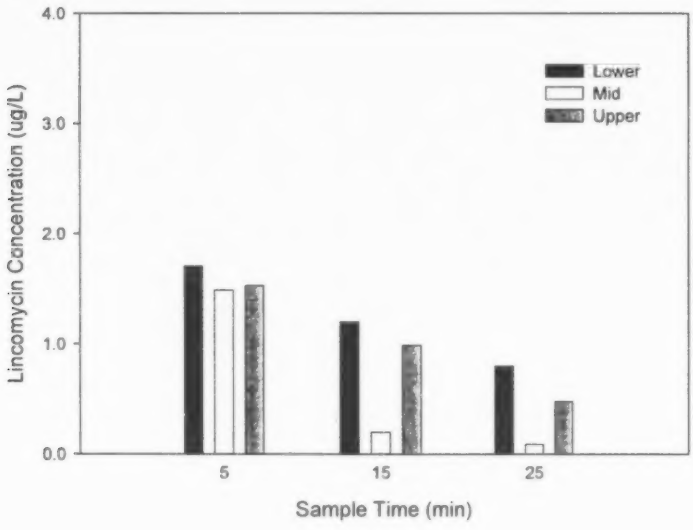


Figure 1c) Lincomycin concentrations (μg L^{-1}) detected in simulated rainfall runoff samples collected from cropland that was manure-treated in fall 2004 at a rate of 78,000 L ha^{-1} at PSCI study site 2. Samples were collected at three different slope positions (upper, mid, lower) in relation to two ephemeral wetlands (i) W1 (ii) W2 and one depression (iii) D1 located on the quarter section.

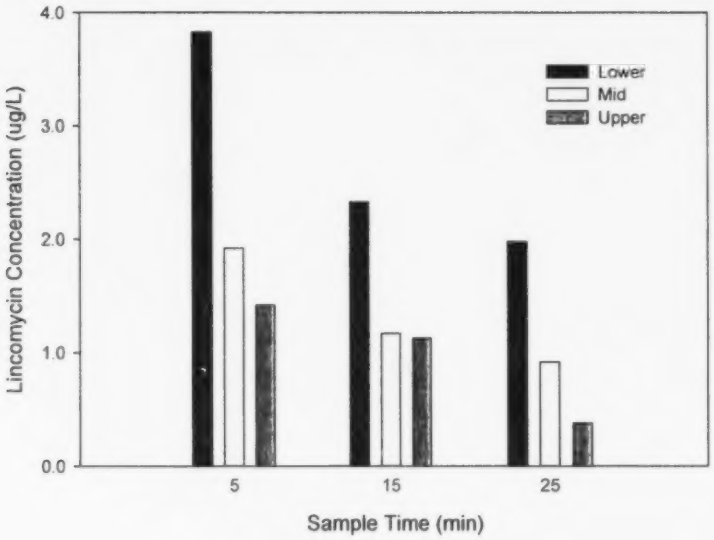
i) W1



ii) W2



iii) D1


4.0
3.0
2.0
1.0
0.0
Lincomycin Concentration (ug/L)
Lower
Mid
Upper
5
15
25
Sample Time (min)

Appendix C

Table 2. Lincomycin concentrations detected in snow melt runoff samples collected from one depression per watershed at PSCI study site 1.

Sample Label	Water Body Type	Date Collected	Manure Application Rate ($L\ ha^{-1}$)	Lincomycin Concentration ($\mu g\ L^{-1}$)
PSCI Study Site 1 - Manure injected fall 2003				
Cereal Stubble				
WS1	depression	31/03/2004	60,000	0.157
		01/04/2004		0.089
		02/04/2004		0.167
		03/04/2004		0.181
		04/04/2004		0.118
		05/04/2004		0.096
		06/04/2004		0.096
		13/04/2004		0.105
		26/04/2004		0.084
WS2	depression	02/04/2004	60,000	0.058
		03/04/2004		0.066
		04/04/2004		0.070
		05/04/2004		0.060
		06/04/2004		0.046
		13/04/2004		0.038
WS3	depression	31/03/2004	95,000	0.802
		01/04/2004		0.636
		02/04/2004		0.600
		03/04/2004		0.328
		04/04/2004		0.414
		05/04/2004		0.545
		06/04/2004		0.307
WS4	depression	02/04/2004	95,000	0.091
		03/04/2004		0.075
		04/04/2004		0.096
		05/04/2004		0.063
		06/04/2004		0.058
		13/04/2004		0.058
WS5	depression	03/04/2004	control field	nd
		04/04/2004		nd
		05/04/2004		nd
		06/04/2004		nd
		07/04/2004		nd
		13/04/2004		nd

WS6	depression	02/04/2004	control field	nd
		05/04/2004		nd
		06/04/2004		nd

Table 3. Lincomycin concentrations detected in snow melt runoff samples collected from ephemeral wetlands at PSCI study site 2.

Sample Label	Water Body Type	Date Collected	Manure Application Rate (L ha^{-1})	Lincomycin Concentration ($\mu g\ L^{-1}$)
PSCI Study Site 2 - Manure injected in fall 2004				
Canola Stubble				
W1	ephemeral wetland	03/04/2005	78,000	363
		05/04/2005		366
		06/04/2005		422
		18/04/2005		275
		25/04/2005		292
W2	ephemeral wetland	01/04/2005	78,000	248
		03/04/2005		228
		05/04/2005		732
		06/04/2005		633
		18/04/2005		440
		25/04/2005		383
W3	ephemeral wetland	01/04/2005	control field	nd
		03/04/2005		nd
		05/04/2005		nd
		06/04/2005		nd
		18/04/2005		nd
		25/04/2005		nd
R1 - W1	runoff	03/04/2005	78,000	629
R2 - W1		03/04/2005	78,000	830
R3 - W2		03/04/2005	78,000	1,014
R4 - W3		03/04/2005	control field	nd

Table 4. Lincomycin concentrations detected in samples collected from depressions and ephemeral wetlands after snow melt runoff at RLSF study site 1.

Sample Label	Water Body Type	Date Collected	Manure Application Rate (L ha^{-1})	Lincomycin Concentration ($\mu g\ L^{-1}$)
RLSF Study Site 1 - Manure injected in fall 2003				
Summerfallow				
SF 1	depression	24/03/2004	79,000	0.011

		01/04/2004		nd
SF 2	depression	24/03/2004	79,000	0.592
		01/04/2004		0.702
		07/04/2004		1.000
		16/04/2004		0.794
SF 3	depression	24/03/2004	79,000	1.659
		01/04/2004		1.714
		07/04/2004		3.205
		16/04/2004		2.305
SF 4	depression	24/03/2004	79,000	1.554
		01/04/2004		1.038
		07/04/2004		1.687
SF 5	depression	24/03/2004	79,000	1.031
SF 6	depression	24/03/2004	79,000	0.907
		01/04/2004		0.715
		07/04/2004		1.797
SF 7	depression	24/03/2004	79,000	1.058
		01/04/2004		0.848
		07/04/2004		1.382
Ctrl 1	depression	24/03/2004	control field	nd
Cereal Stubble				
ST 1	depression	24/03/2004	79,000	4.192
ST 2	depression	24/03/2004	79,000	3.291
ST 3	depression	24/03/2004	79,000	1.392
ST 4	depression	01/04/2004	79,000	0.277
ST 5	depression	24/03/2004	79,000	0.024
ST 6	depression	24/03/2004	79,000	1.773
ST 7	depression	24/03/2004	79,000	1.064
		01/04/2004		1.182
ST 8	depression	24/03/2004	79,000	2.354

Table 5. Lincomycin concentrations detected in samples collected from depressions and ephemeral wetlands after snow melt

runoff at RLSF study site 2.

Sample Label	Water Body Type	Date Collected	Manure Application Rate (L ha^{-1})	Lincomycin Concentration ($\mu g\ L^{-1}$)
RLSF Study Site 2 - Centre pivot manure application in fall 2003				
Cereal Stubble				
NW 1	depression	24/03/2004	3,000	0.753
NW 2	depression	24/03/2004	3,000	1.396
NW 3	depression	01/04/2004	3,000	0.938
NW 4	depression	24/03/2004	3,000	1.160
NW 5	depression	01/04/2004	3,000	1.300
NW 6	depression	24/03/2004	3,000	0.948
NW 7	depression	24/03/2004	3,000	nd
NW 8	depression	24/03/2004	3,000	1.027
Canola Stubble				
NE 1	depression	24/03/2004	3,000	1.547
NE 2	depression	24/03/2004	3,000	0.295
		01/04/2004		0.639
NE 3	ephemeral wetland	24/03/2004	3,000	0.155
		01/04/2004		0.702
		07/04/2004		0.825
NE 4	depression	24/03/2004	3,000	2.214
NE 5	ephemeral wetland	24/03/2004	3,000	0.385
		01/04/2004		0.168
		07/04/2004		0.334
		16/04/2004		0.346
		23/04/2004		0.726
NE 6	ephemeral wetland	24/03/2004	3,000	0.486
		01/04/2004		0.238
		07/04/2004		0.296
		16/04/2004		0.690
		23/04/2004		0.589

NE 7	depression	24/03/2004	3,000	0.410
NE 8	depression	24/03/2004	3,000	0.212
		01/04/2004		0.131
NE 9	depression	24/03/2004	3,000	0.901
NE 10	depression	01/04/2004	3,000	1.609
NE 11	depression	01/04/2004	3,000	0.542
NE 12	depression	01/04/2004	3,000	0.455
NE 13	depression	01/04/2004	3,000	21.65
Ctrl 2	depression	24/03/2004	control field	nd
Ctrl 3	depression	24/03/2004	control field	nd
		01/04/2004		nd
Ctrl 4	depression	01/04/2004	control field	nd
Ctrl 5	depression	01/04/2004	control field	nd
Ctrl 6	depression	01/04/2004	control field	nd

Table 6. Lincomycin concentrations detected in samples collected from depressions and ephemeral wetlands after snow melt runoff at RLSF study site 3.

Sample Label	Water Body Type	Date Collected	Manure Application Rate (L ha^{-1})	Lincomycin Concentration ($\mu g\ L^{-1}$)
RLSF Study Site 3 - Spring application, manure injected in spring 2004				
Canola Stubble				
SW 1	depression	31/03/2005	90,000	0.005
		05/04/2005		0.008
SW 2	depression	31/03/2005	90,000	0.008
		05/04/2005		0.148
SW 3	depression	31/03/2005	90,000	0.010
SW 4	ephemeral wetland	31/03/2005	90,000	0.003
		05/04/2005		0.026
		13/04/2005		0.002
		21/04/2005		0.007

		28/04/2005		0.006
SW 5	ephemeral wetland	31/03/2005	90,000	0.006
		05/04/2005		0.025
		13/04/2005		nd
		21/04/2005		nd
		28/04/2005		0.023
Canola Stubble				
SE 6	ephemeral wetland	31/03/2005	90,000	0.008
		05/04/2005		0.007
		13/04/2005		0.002
		21/04/2005		0.003
		28/04/2005		0.006
SE 7	depression	31/03/2005	90,000	0.005
		05/04/2005		0.029
SE 8	ephemeral wetland	31/03/2005	90,000	0.007
		05/04/2005		0.112
		13/04/2005		nd
		21/04/2005		0.004
		28/04/2005		0.016
SE 9	depression	31/03/2005	90,000	0.004
		05/04/2005		0.025
		21/04/2005		0.006
SE 10	depression	31/03/2005	90,000	0.005
SE 11	depression	31/03/2005	90,000	0.004
		05/04/2005		0.007
		21/04/2005		0.009
		28/04/2005		0.031

Table 7. Lincomycin concentrations detected in samples collected from depressions, ephemeral wetlands and dugouts after snow melt runoff at RLSF study site 4.

Sample Label	Water Body Type	Date Collected	Manure Application Rate (L ha^{-1})	Lincomycin Concentration ($\mu g\ L^{-1}$)
RLSF Study Site 4 - Manure injected in fall 2004				
Pasture Land				
SW-9 1	depression	08/03/2005	88,000	0.005
SW-9 2	depression	08/03/2005	88,000	0.149

		29/03/2005		0.298
		31/03/2005		0.181
SW-9 3	depression	08/03/2005	88,000	0.270
		29/03/2005		0.595
SW-9 4	dugout	08/03/2005	88,000	0.201
		29/03/2005		0.044
		05/04/2005		0.168
		21/04/2005		0.077
		28/04/2005		0.085
SW-9 5	depression	08/03/2005	88,000	0.152
		29/03/2005		0.128
		31/03/2005		0.142
SW-9 6	depression	08/03/2005	88,000	0.471
		29/03/2005		0.199
		31/03/2005		0.214
		05/04/2005		0.164
		13/04/2005		0.137
		21/04/2005		0.341
SW-9 7	ephemeral wetland	31/03/2005	88,000	0.393
		05/04/2005		0.318
SW-9 8	depression	31/03/2005	88,000	0.119
		05/04/2005		0.211
		13/04/2005		0.179
SW-9 9	ephemeral wetland	31/03/2005	88,000	0.116
		05/04/2005		0.103
		13/04/2005		0.087
		21/04/2005		0.071
		28/04/2005		0.101
SW-9 10	depression	08/03/2005	88,000	0.406
SW-9 11	depression	29/03/2005	88,000	0.484
SW-9 12	ephemeral wetland	31/03/2005	88,000	0.075
		05/04/2005		0.098
		21/04/2005		0.104
		28/04/2005		0.215
SW-9 13	ephemeral wetland	31/03/2005	88,000	0.250
		05/04/2005		0.178

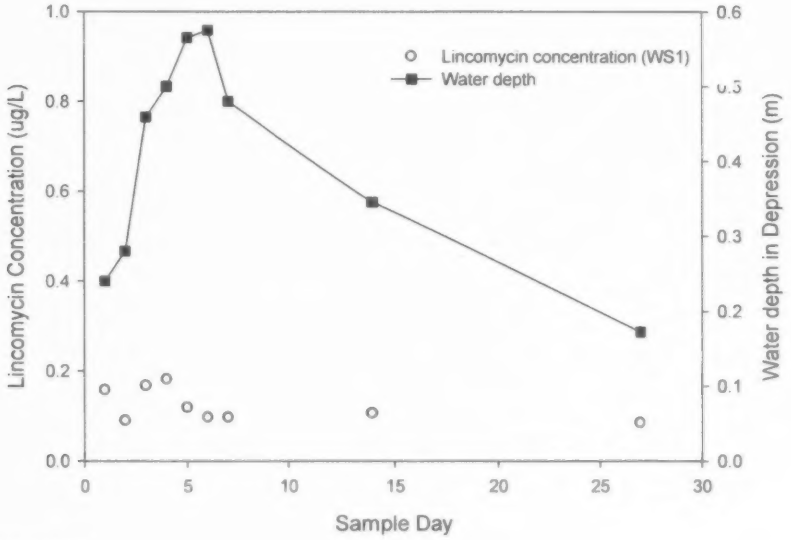
		13/04/2005		0.147
		21/04/2005		0.176
		28/04/2005		0.144
SW-9 14	depression	31/03/2005	88,000	0.318
		05/04/2005		0.563
		13/04/2005		0.084
		21/04/2005		0.413
		28/04/2005		0.361
Pasture Land				
NW-4 1	depression	31/03/2005	110,000	0.120
NW-4 2	depression	31/03/2005	110,000	0.566
		05/04/2005		0.235
NW-4 3	depression	31/03/2005	110,000	0.124
		05/04/2005		0.129
		13/04/2005		0.036
		21/04/2005		0.058
		28/04/2005		0.118
NW-4 4	dugout	29/03/2005	110,000	0.276
		31/03/2005		0.222
		05/04/2005		0.225
		13/04/2005		0.202
		21/04/2005		0.171
		28/04/2005		0.164
NW-4 5	depression	31/03/2005	110,000	0.273
NW-4 6	depression	29/03/2005	110,000	0.214
		31/03/2005		0.720
		05/04/2005		0.485
NW-4 7	depression	29/03/2005	110,000	0.827
		31/03/2005		0.500
		05/04/2005		0.455
NW-4 8	depression	29/03/2005	110,000	0.441
		31/03/2005		0.395
		05/04/2005		0.395
		13/04/2005		0.439
		21/04/2005		0.505
		28/04/2005		0.531
NW-4 9	depression	29/03/2005	110,000	0.391

		31/03/2005		0.375
		05/04/2005		0.381
NW-4 10	depression	31/03/2005	110,000	0.335
NW-4 11	depression	31/03/2005	110,000	0.421
NW-4 12	depression	29/03/2005	110,000	0.844
		31/03/2005		0.473
		05/04/2005		0.375
NW-4 13	depression	29/03/2005	110,000	0.398
		31/03/2005		0.385
		05/04/2005		0.399

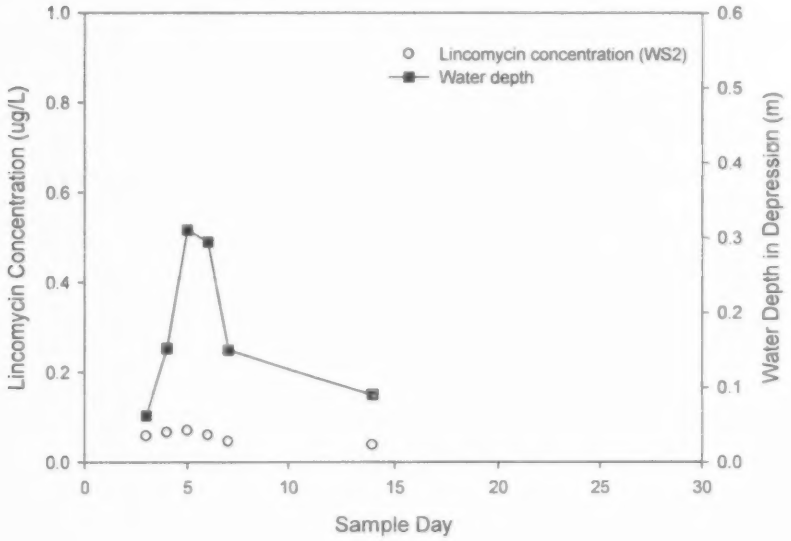
Appendix D

Figure 2a) Lincomycin concentrations (µg L^{-1}) detected in samples collected from depressions located within each watershed (i) WS1 (ii) WS2 (iii) WS3 (iv) WS4 at PSCI study site 1. Cropland was manure-treated at two application rates (60,000 and 95,000 L ha^{-1}) in the fall of 2003. Snow melt runoff samples were collected in the spring of 2004. Water depth in each depression was also monitored.

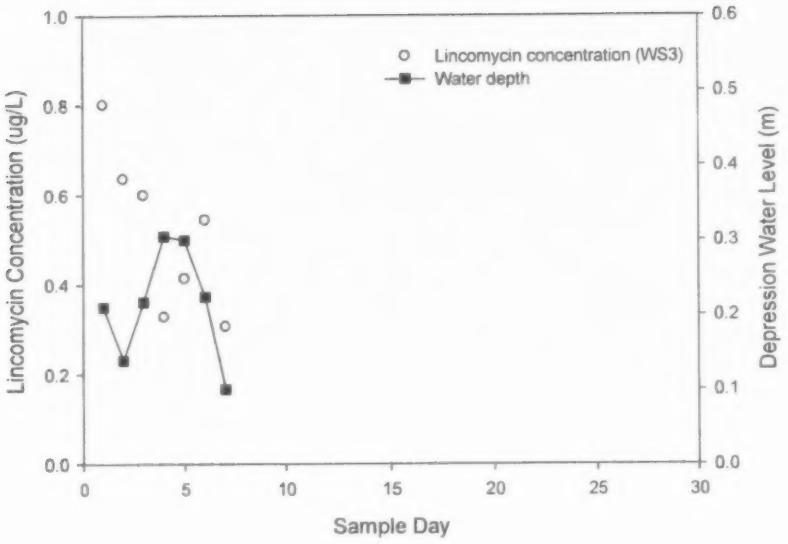
i) WS1



ii) WS2



iii) WS3



iv) WS4

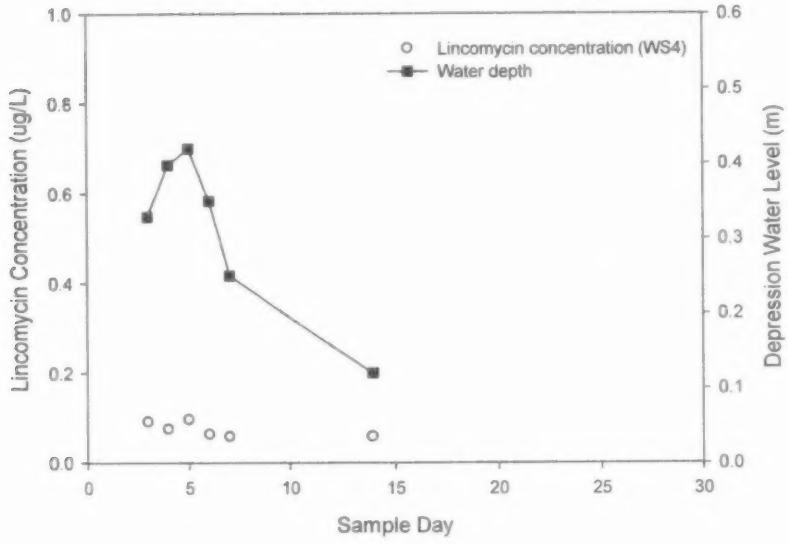


Figure 2b) Lincomycin concentrations (μg L^{-1}) detected in samples collected from two ephemeral wetlands (i) W1 (ii) W2 at PSCI study site 2. Cropland was manure-treated at 78,000 L ha^{-1} in the fall of 2004. Snow melt runoff samples were collected in the spring of 2005.

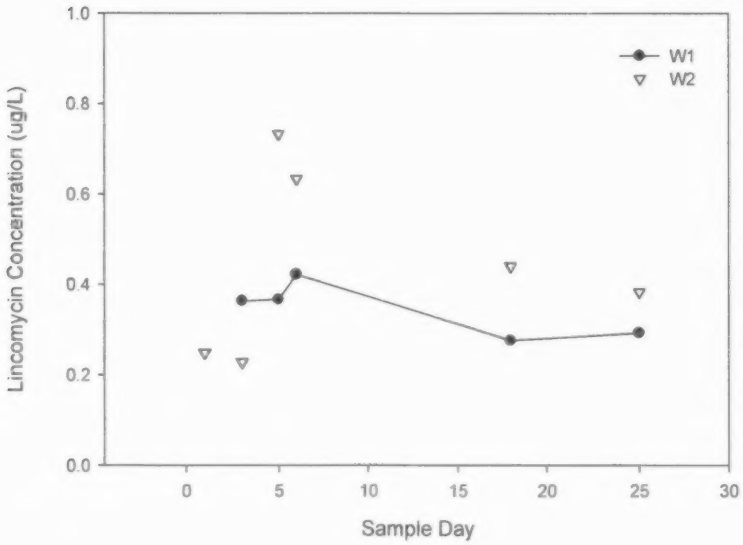


Figure 2c) Lincomycin concentrations (μg L^{-1}) detected in samples collected from depressions on summerfallow which was manure-treated at 79,000 L ha^{-1} in the fall of 2003 (RLSF study site 1). Snow melt runoff samples were collected in the spring of 2004.

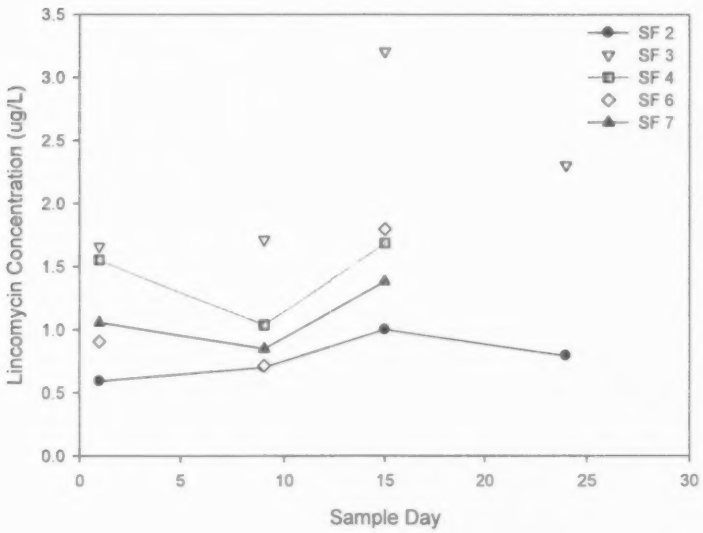


Figure 2d) Lincomycin concentrations (μg L^{-1}) detected in samples collected from ephemeral wetlands on cropland which was manure-treated using a centre pivot at 3,000 L ha^{-1} in the fall of 2003 (RLSF study site 2). Snow melt runoff samples were collected in the spring of 2004.

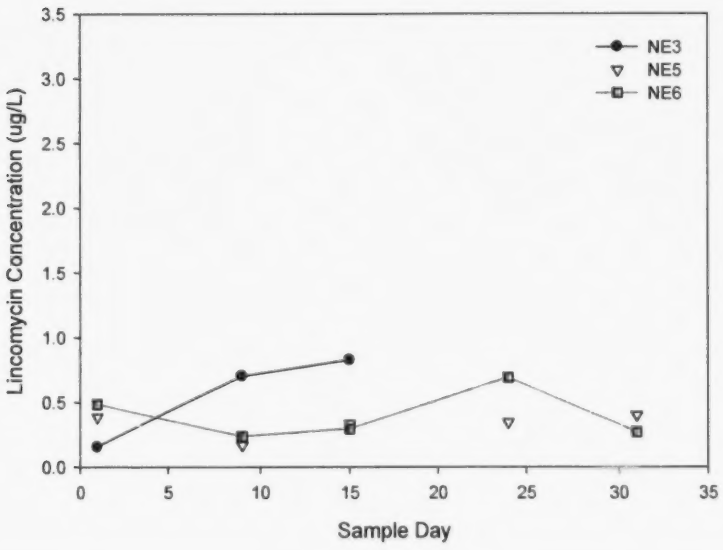
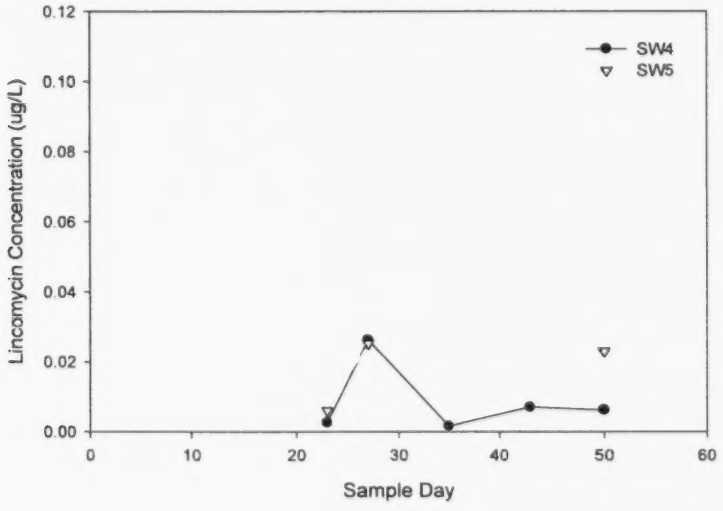
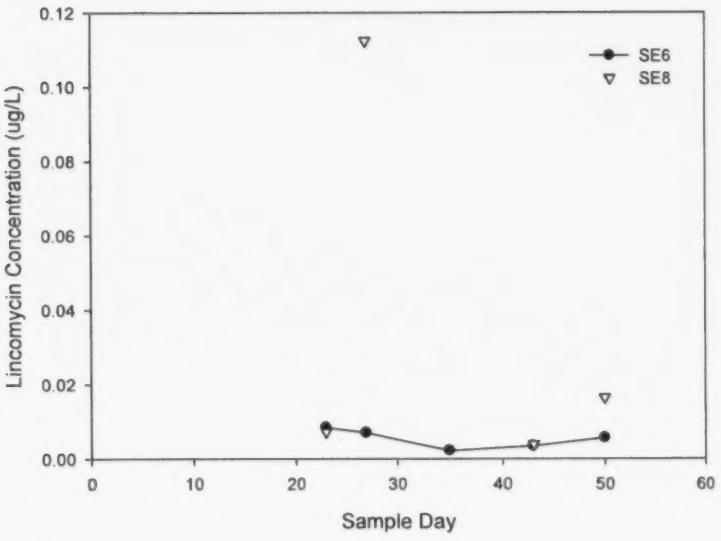


Figure 2e) Lincomycin concentrations (μg L^{-1}) detected in samples collected from ephemeral wetlands (i) SW4, SW5 (ii) SE6, SE8 and depressions (iii) SE9 and SE11 on cropland which was manure-treated in the spring of 2004 at 90,000 L ha^{-1} (RLSF study site 3). Snow melt runoff samples were collected a year later in the spring of 2005.

i) ephemeral wetlands, SW quarter section



ii) ephemeral wetlands, SE quarter section



iii) depressions, SE quarter section

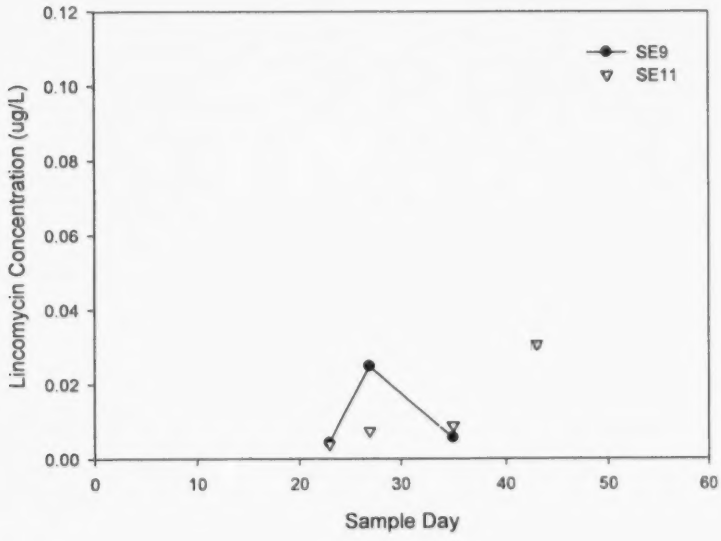
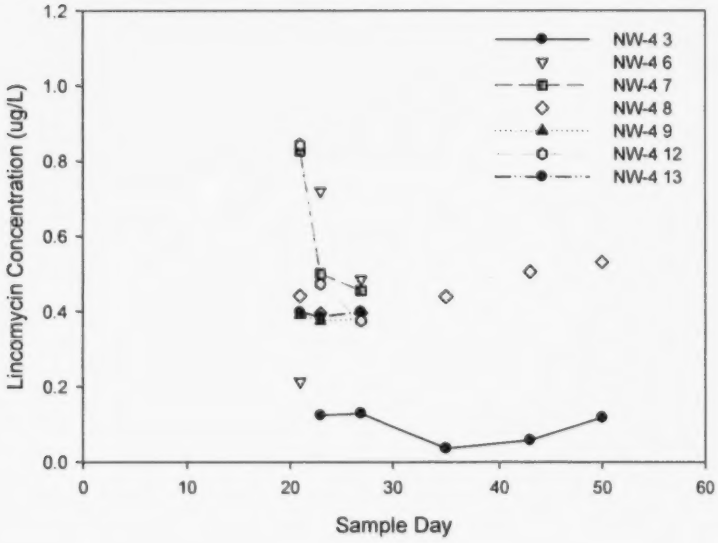
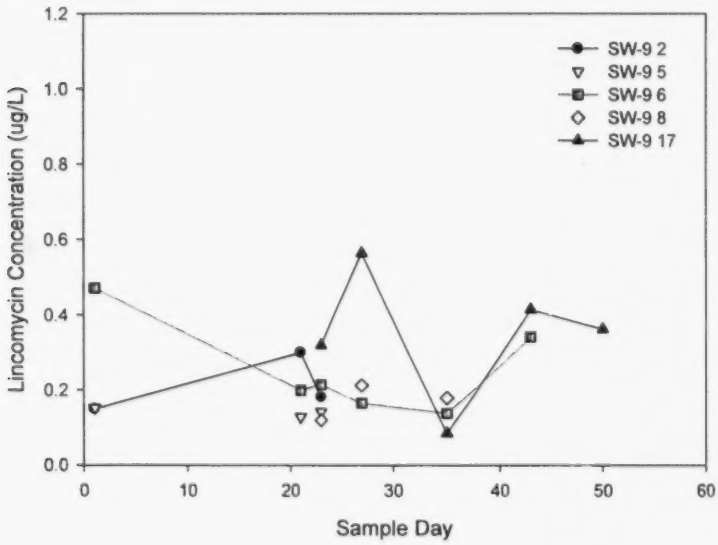


Figure 2f) Lincomycin concentrations (µg L^{-1}) detected in samples collected from depressions (i) NW-4 3, NW-4 6, NW-4 7, NW-4 8, NW-4 9, NW-4 12, NW-4 13 (ii) SW-9 2, SW-9 5, SW-9 6, SW-9 8, SW-9 14, ephemeral wetlands (iii) SW-9 9, SW-9 12, SW-9 13, and dugouts (iv) NW-4 4 and SW-9 4, from RLSF study site 4. Cropland was manure-treated at 110,000 and 88,000 L ha^{-1} on the NW-4 and SW-9 quarter sections, respectively, in the fall of 2004. Snow melt runoff samples were collected in the spring of 2005.

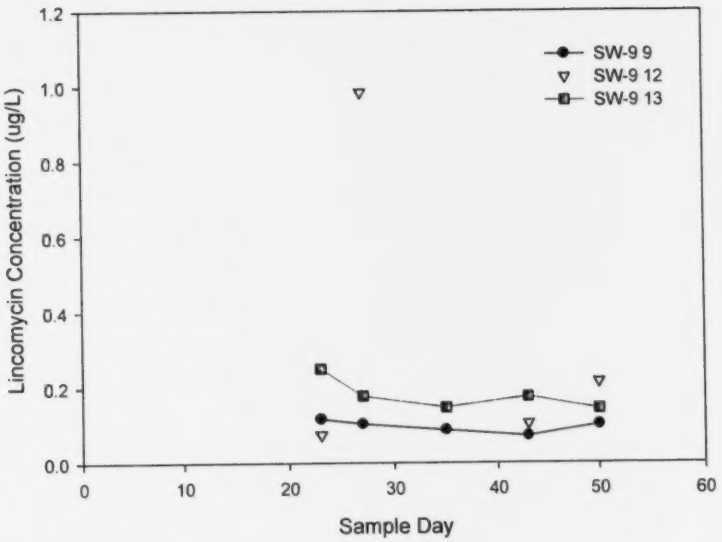
i) NW-4 depressions



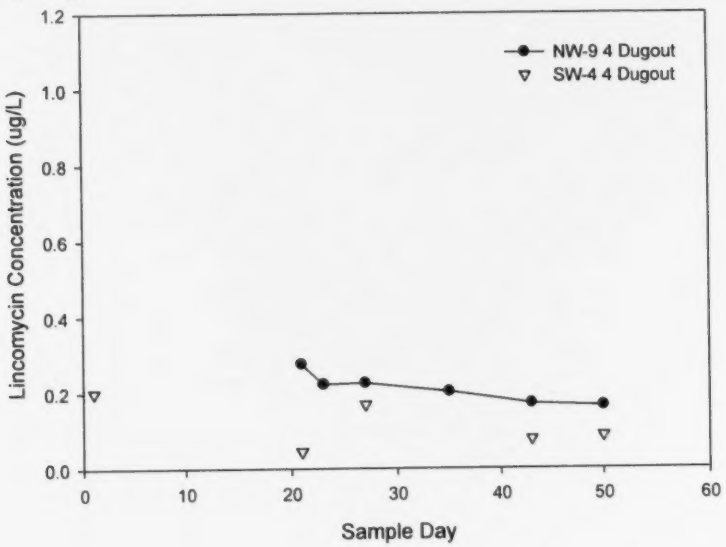
ii) SW-9 depressions



iii) SW-9 ephemeral wetlands



iv) Dugouts



Appendix E

Table 8. Lincomycin concentrations detected in groundwater collected from a single piezometer per watershed located at the centre of a depression. There were two manure application rates (60,000 and 95,000 L/ha) at this study site in fall 2003, previously the fields were manure treated in 2001 or 2002.

Sample	Piezometer Depth (m)	Date Collected	Previous Manure Application Year	Manure Application Rate in fall 2003 ($L\ ha^{-1}$)	Lincomycin Concentration ($\mu g\ L^{-1}$)
WS1	4.1	02/10/2003	2001	60,000	nd
	4.1	06/05/2004	2001	60,000	nd
		12/05/2004			0.0002
		02/06/2004			0.0008
		17/06/2004			nd
		30/06/2004			nd
		08/07/2004			nd
		29/07/2004			0.0002
		30/08/2004			nd
	4.1	31/03/2005	2001	60,000	0.0008
		18/04/2005			0.0004
		25/04/2005			ns
		09/05/2005			0.0004
		17/06/2005			0.0009
		24/06/2005			ns
		19/07/2005			0.0006
		21/08/2005			nd
		25/09/2005			nd
		08/10/2005			nd
WS2	4.81	02/10/2003	2001	60,000	ns
	4.81	30/06/2004	2001	60,000	nd
		08/07/2004			nd
		29/07/2004			nd
	4.81	31/03/2005	2001	60,000	0.0010
		18/04/2005			ns
		25/04/2005			0.0028
		09/05/2005			0.0002
		17/06/2005			0.0013
		24/06/2005			0.0019
		19/07/2005			0.0002
		21/08/2005			ns

		25/09/2005			ns
		08/10/2005			ns
WS3	4.02	02/10/2003	2001	95,000	ns
	4.02	30/06/2004	2001	95,000	nd
		08/07/2004			nd
	4.02	31/03/2005	2001	95,000	0.0004
		18/04/2005			0.0008
		25/04/2005			0.0009
		09/05/2005			0.0018
		17/06/2005			0.0008
		24/06/2005			0.0008
		19/07/2005			0.0003
		21/08/2005			ns
		25/09/2005			ns
		08/10/2005			ns
WS4	5.37	02/10/2003	2001	95,000	ns
	5.37	02/06/2004	2001	95,000	0.1540
		30/06/2004			0.0142
		08/07/2004			0.0068
		29/07/2004			0.0040
		30/08/2004			0.0022
	5.37	31/03/2005	2001	95,000	0.0013
		18/04/2005			0.0008
		25/04/2005			ns
		09/05/2005			0.0006
		17/06/2005			0.0028
		24/06/2005			0.0010
		19/07/2005			0.0005
		21/08/2005			nd
		25/09/2005			nd
		08/10/2005			nd
Control	3.85	02/10/2003	2002	control field	nd
	3.85	06/05/2004	2002	control field	nd
		12/05/2004			nd
		03/06/2004			nd
		17/06/2004			nd
		30/06/2004			nd

	08/07/2004			nd
	29/07/2004			nd
	30/08/2004			nd
3.85	31/03/2005	2002	control field	0.0015
	18/04/2005			0.0005
	25/04/2005			0.0009
	09/05/2005			0.0003
	17/06/2005			0.0003
	24/06/2005			ns
	19/07/2005			0.0004
	21/08/2005			nd
	25/09/2005			nd
	08/10/2005			nd

Project # 20020079: Persistence of Antibiotics in Liquid Hog Manure Applied to Cropland and their Transport in Surface Runoff

Additional Notes:

The focus of research project # 20020079 was to determine antibiotic degradation and transport in liquid hog manure to the surrounding ecosystem after application. This is exploratory work that was initiated in 2003.

The two antibiotics in question were spectinomycin and lincomycin, which are administered to prevent post-weaning diarrhoea in pigs. They are commonly used antibiotics in the intensive hog industry (Rajic et al., 2006; Wagner, 2006), and it is important to attain information regarding their fate in the environment.

This report identifies that spectinomycin was not present at detectable limits, however lincomycin was detected in manure, soil, surface and ground water samples. Lincomycin resisted degradation and it would be useful to support further research into, at what concentrations, if any, this antibiotic may have an impact on the surrounding environment. Alan Cessna is continuing work on solid cattle manure and antibiotic transport. He is also partnering with other researchers to look into the biologic effects of antibiotics in the ecosystem, as well as their persistence in both water columns and sediments of surface waters. This is a relatively new area of research in Saskatchewan, and continues to develop in both analytical methodology and understanding of the factors that influence antibiotic persistence.

Rajic, Andrijana, Richard Reid-Smith, Ann Deckert, Catherine Dewey, Scott McEwen. 2006. Reported antibiotic use in 90 swine farms in Alberta. Can. Vet. J. 47: 446-452.

Harvey Wagner. Manager of Producer Services, SaskPork.
Personal Communications with both Harvey and Ernie Donnawell, Feb. 7th, 2007.
Harvey Wagner, Phone: 306-343-3502
Email: hwagner@saskpork.com

Reported antibiotic use in 90 swine farms in Alberta

Andrijana Rajić, Richard Reid-Smith, Anne E. Deckert, Catherine E. Dewey, Scott A. McEwen

Abstract — Antibiotic use was described using a convenience sample of 90 Alberta swine farms representing approximately 25% of the Alberta market swine production. Data on the use of antibiotics were collected through an on-farm interview questionnaire. The vast majority of antibiotics were used in feed. The chlortetracycline/sulfamethazine/penicillin combination and tylosin were the most frequently used in-feed antibiotics in weaners and growers/finishers, respectively. The use of antibiotics through water was reported mostly occasionally in all categories. The use of injectable antibiotics was reported mostly in sick pigs. Penicillin was the most common in-water and injectable antibiotic in all categories. The apparent low frequency of critically important antimicrobials for use in humans (quinolones and 3rd generation cephalosporins) is an encouraging finding from a public health perspective. The widespread and frequently reported use of penicillin and tetracycline are of public health concern considering that both antimicrobials are also used for therapeutic purposes in human medicine.

Résumé — Rapport sur l'utilisation d'antibiotiques dans 90 porcheries de l'Alberta. L'utilisation d'antibiotiques a été décrite à l'aide d'un échantillon pratique de 90 porcheries d'Alberta représentant approximativement 25 % du marché albertain de la production porcine. Les données sur l'utilisation des antibiotiques ont été recueillies via un questionnaire rempli lors d'un entretien réalisé sur les lieux. La grande majorité des antibiotiques étaient administrés dans la nourriture. L'association chlortétracycline/sulfaméthazine/pénicilline et la tylosine étaient les plus fréquemment utilisés dans les aliments chez les naisseurs et les engraisseurs/finisseurs respectivement. L'utilisation d'antibiotiques dans l'eau a été rapportée, le plus souvent occasionnellement, dans toutes les catégories. L'utilisation d'antibiotiques injectables a été rapportée le plus souvent chez les porcs malades. La pénicilline était l'antibiotique le plus fréquemment utilisé dans l'eau ou en injection. La faible fréquence d'utilisation d'antimicrobiens d'importance cruciale chez l'homme (quinolones et céphalosporines de 3ième génération) est un fait encourageant du point de vue de la santé publique. L'étendue et la fréquence rapportée de l'utilisation de pénicilline et de tétracycline constitue une préoccupation pour la santé publique du fait que ces 2 antibiotiques sont également utilisés à des fins thérapeutiques en médecine humaine.

(Traduit par Docteur André Blouin)

Can Vet J 2006;47:446–452

Introduction

Antibiotic use in food animals is associated with substantial benefits, but also with potential public health risks (1–10). In swine, antibiotics are used to treat and prevent disease in swine, to protect swine welfare, and to improve growth rate and efficiency of feed utilization (1–2,9–10). The potential public health risks related to the use of antibiotics in swine and other food animals include the contamination of food with drug residues, release of drug residues into the environment, the selection for antibiotic resistant bacteria, and an occupational exposure of farmers and others to drugs (1–3,11). Although much of the antibiotic resistance observed in important human pathogens may be ascribed to use of antibiotics in human medicine, the use of antibiotics in agri-food production is also believed to contribute to this global public health problem (1,3–7).

Appropriate assessments of the selective pressure imposed by various types of antibiotic use in food animals are necessary to understand and anticipate antibiotic resistance trends in human and animal populations and in the environment (1,12–14). Periodic audits and statistically based surveys of end-users of food animal antibiotics, namely veterinarians and livestock producers, are suggested as relevant sources of information regarding the use of antibiotics in food animal production (15).

There is little published farm-level information about antibiotic use in food animals. In Canada, the use of

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antibiotics in swine has been described most extensively in Ontario (16–17). However, swine production systems differ substantially among provinces, so the information from Ontario may not be representative of that for the entire Canadian swine population. No Canadian study conducted to date has investigated the use of antibiotics in western Canada, using a farm-based questionnaire. Thus, the purpose of this study was to describe the antibiotic use patterns and the types of antibiotics used through various phases of production reported by 90 swine operations in Alberta. Collection of information on specific dosing regimes was beyond the scope of the study.

Materials and methods

General aspects of the study

A study was conducted in Alberta from May to September 2000, with the objective of determining the prevalence and serovar diversity of *Salmonella* spp. in finisher pigs and the risk factors for *Salmonella* infections on 90 swine finisher farms. Methods utilized for the selection of these farms have been described in detail elsewhere (18). In brief, 10 veterinary practitioners selected 90 swine farms in Alberta, based on an annual production of ≥ 2000 market pigs per farm and the willingness of the producers to participate in the study.

Data on antibiotic use were collected by using a questionnaire designed to determine the management practices of participating farms. The questionnaire (copy available from the first author upon request) comprised 4 sections: production system and management, antibiotic use, health status, and biosecurity. Multisite operation was defined as a corporate enterprise with ≥ 2 sites. Other operations (farrow-to-finish, farrow-to-wean, individual grow-to-finish, or finishing farms) referred to owner operated facilities. The questionnaire was pretested by 3 pork producers, 3 provincial pork extension specialists, and 3 veterinarians who were familiar with the survey technique. The owner or operator of the farm completed the questionnaire with his or her herd veterinarian.

Collection of antibiotic use and farm health information

The questionnaire included a 4-page section on the use of antibiotics consisting of 3 subsections. In the 1st subsection, respondents were asked to indicate for weaners, growers, and finishers the percentage (%) of time that antibiotics were added to feed during the past 12 mo (summer 1999 to summer 2000) by using the following response options: never, occasionally (< 50%), frequently (50% to 95%) and always (> 95%). They were also asked to specify, from a list of trade and generic names, the types of in-feed antibiotics used in feeds of weaners, growers, and finishers, during the past 12 mo. In the 2nd subsection, respondents were asked to indicate the percentage of time for weaners, growers, finishers, and sows that antibiotics were added to water during the past 12 mo by using the same response options as described above. They were also asked to specify, from a list of trade and generic names, the types of in-water antibiotics used for those categories during the past 12 mo. In the 3rd subsection, respondents were asked to indicate, from a list of trade and generic names, the types of specific injectable antibiotics used during the past 12 mo for weaners, growers, and finishers by using the following response options: whole barn, whole pen, and sick pigs only.

Table 1. Number (%) of farms in which any antibiotic use was reported through feed, water, and injection in various phases of production in 90 swine farms in Alberta in 2000

Antibiotic route of administration	Number (%) of farms administering antibiotics in this fashion during the past 12 mo		
	Weaners[a]	Growers[b]	Finishers[c]
Feed	76 (100) (95%–100%)[d]	80 (90.9) (83%–96%)[d]	72 (80.0) (70%–88%)[d]
Water	38 (50.0) (38%–62%)[d]	18 (20.5) (13%–30%)[d]	16 (17.8) (11%–27%)[d]
Injection	65 (85.5) (76%–93%)[d]	62 (70.5) (60%–80%)[d]	50 (55.6) (45%–66%)[d]

[a]Weaning phase of 76 farrow-to-finish farms
[b]Growing phase of 76 farrow-to-finish farms, 6 individual grow-to-finish farms, and 6 grow-to-finish farms within multisite operations
[c]Finishing phase of 76 farrow-to-finish farms, 6 individual grow-to-finish farms, 6 grow-to-finish farms within multisite operations, and 2 finishing farms
[d]95% confidence intervals

A 1-page section on the disease status of the herd included a list of 16 diseases and their respective agents that are frequently observed on commercial swine farms in North America. Respondents were asked to indicate which of those diseases had been diagnosed in the various pig categories by the herd veterinarian, diagnostic laboratory, or both, during the 12 mo preceding the survey by using the following response options: never, occasionally, and commonly.

Data manipulation and statistical analyses

Questionnaire data were entered into a spreadsheet program (Microsoft Excel 2000; Microsoft Corporation, Redmond, Washington, USA). Data were verified for accuracy by checking each entry against the original hard copy data and then transferred into a statistical software package (Statistix version 2000; STATISTIX Analytical Software, St. Paul, Minnesota, USA) for descriptive statistical analyses. The frequencies and percentages of farms reporting the use of antibiotics through feed, water, and injection were computed for various production phases. Descriptive measures were also computed for the types of antibiotics that were reported to have been administered through feed, water, and injection in various production phases during the previous 12 mo. Confidence intervals for proportions were obtained by using the exact binomial distributions option of a statistical software program (Stata Intercooled, version 8; Stata Corporation, College Station, Texas, USA).

Results

Brief farm description

The annual production of the farms studied represented approximately 25% of the market swine production in Alberta. Participating farms were geographically

Table 2. The most frequent in-feed antibiotic use patterns reported by 76 farrow-to-finish swine farms in Alberta in 2000[a]

Number of farms (%)	% of time antibiotics added to feed during the past 12 mo											
	Weaners				Growers				Finishers			
	0	< 50	50–95	> 95	0	< 50	50–95	> 95	0	< 50	50–95	> 95
47 (61.8)				+				+				+
8 (10.5)				+				+			+	
8 (10.5)				+				+	+			
6 (7.8)				+	+				+			

[a]Other in-feed antibiotic use patterns were reported by ≤ 2 participating farrow-to-finish farms

representative of the major swine production areas in Alberta. Seventy-six (84.4%) farms were farrow-to-finish, while 6 (6.7%), 6 (6.7%), and 2 (2.2%) farms were multisite operations, grow-to-finish and finisher, respectively. Among farrow-to-finish and multisite operations, the number of sows per farm ranged from 100 to 3000 (mean = 525, median = 300). The information on the disease status was incomplete, because 3 practitioners declined to complete that section of the questionnaire for 24 participating farms for privacy reasons. Therefore, no statistical analysis was attempted to evaluate if reported disease status was associated with reported antibiotic use at the farm level.

Antibiotic use in feed

Table 1 shows the proportions of farms in which the use of antibiotics through feed was reported in various phases of production. Ten antibiotic in-feed use patterns were reported at the farm level among the 76 farms that raised weaners, growers, and finishers. The most frequent antibiotic use patterns among the 76 farrow-to-finish farms are shown in Table 2.

Weaners

The use of antibiotics through feed was reported > 95%, between 50% and 95%, and < 50% of the time in 73 (96.1%), 1 (1.3%), and 2 (2.6%) farrow-to-finish farms, respectively. The use of 1, 2, 3, 4, and 5 in-feed antibiotics was reported in 16 (21.1%), 8 (10.5%), 40 (52.6%), 8 (10.5%), and 3 (3.9%) farms, respectively. Two antibiotic drug combinations and 8 individual antibiotics were reported (Table 3).

Growers

The use of antibiotics through feed was reported > 95% of the time, between 50% and 95%, < 50%, and none of the time in 75 (85.2%), 3 (3.4%), 2 (2.3%), and 8 (9.1%) farms, respectively. The use of 1, 2, 3, 4, and 6 in-feed antibiotics was reported in 49 (55.7%), 22 (25.0%), 1 (1.1%), 7 (7.9%), and 1 (1.1%) farms, respectively. Two antibiotic drug combinations and 8 individual antibiotics were reported (Table 3).

Finishers

Antibiotic use through feed was reported > 95% of the time, between 50% and 95%, < 50%, and none of the time in 54 (60%), 13 (14.4%), 4 (4.4%), and 18 (20%) farms, respectively. The use of 1, 2, and 3 in-feed antibiotics was reported in 57 (63.3%), 13 (14.4%), and 1 (1.1%) farms, respectively. One antibiotic drug combination and 6 individual antibiotics were reported (Table 3).

Sows

The use of 1 in-feed antibiotic during the past 12 mo was reported most frequently. Two in-feed antibiotic drug combinations and 7 individual antibiotics were reported in lactating sows, whereas 1 in-feed antibiotic drug combination and 7 individual antibiotics were reported in dry sows (Table 3).

Antibiotic use in water

Table 1 shows the proportion of farms in which the use of antibiotics through water was reported in various production phases during the past 12 mo. Among these farms, mostly occasional antibiotic use was reported in all phases of production.

Weaners

The use of 1, 2, and 3 in-water antibiotics was reported in 25 (32.9%), 11 (14.5%), and 3 (3.9%) farms, respectively. Four antibiotic drug combinations and 4 individual antibiotics were reported (Table 4).

Growers

The use of 1 and 2 in-water antibiotics was reported in 15 (17.0%) and 3 (3.4%) farms, respectively. One antibiotic drug combination and 5 individual antibiotics were administered through water (Table 4).

Finishers

The use of 1 and 2 in-water antibiotics was reported in 14 (15.6%) and 2 (2.2%) farms, respectively. Four individual antibiotics were reported (Table 4).

Sows

In dry and lactating sows, only occasional use of antibiotics through water was reported in 4 (5.3%) and 1 (1.3%) farms, respectively, during the past 12 mo. Only 2 antibiotics were reported in these categories (Table 4).

Antibiotic use through injection

Table 1 gives the proportion of farms in which the use of antibiotics through injections was reported in various phases of production during the past 12 mo. One antibiotic drug combination and 5 individual antibiotics were reported in all production phases (Table 5). The use of injectable antibiotics limited to sick pigs only was reported in all phases of production. In weaners, the use

Table 3. Reported type of antibiotic used through feed in various phases of production in 90 swine farms in Alberta in 2000

Type of antibiotic	Number of farms (%) using this antibiotic				
	Weaners[a]	Growers[b]	Finishers[c]	L. Sows[d]	D. Sows[e]
Bacitracin	Ø	Ø	Ø	Ø	Ø
Carbadox	16 (21.1)[f] (13%–32%)[f]	4 (4.5) (1.3%–11%)[f]	Ø	Ø	Ø
Chlortetracycline	7 (9.2) (4%–18%)[f]	10 (11.4) (6%–20%)[f]	5 (5.6) (1.8%–12.4%)[f]	7 (9.2) (4%–18%)[f]	8 (10.5) (5%–20%)[f]
Chlortetracycline/ sulfamethazine/penicillin	45 (59.2) (47%–70%)[f]	5 (5.7) (1.9%–13%)[f]	Ø	6 (7.9) (3%–16%)[f]	3 (3.9) (0.8%–11%)[f]
Dimetridazole	1 (1.3) (0.03%–7%)[f]	7 (7.9) (3.2%–16%)[f]	Ø	6 (7.9) (3%–16%)[f]	8 (10.5) (5%–20%)[f]
Lincomycin	6 (7.9) (3%–16%)[f]	30 (34.1) (24%–45%)[f]	19 (21.1) (13%–31%)[f]	1 (1.3) (0.03%–7%)[f]	2 (2.6) (0.3%–9%)[f]
Lincomycin/ spectinomycin	12 (15.8) (8%–26%)[f]	1 (1.1) (0.03%–6%)[f]	2 (2.2) (0.3%–8%)[f]	1 (1.3) (0.03%–7%)[f]	Ø
Oxytetracycline	4 (5.3) (1.5%–13%)[f]	9 (10.2) (5%–19%)[f]	3 (3.3) (0.7%–9%)[f]	3 (3.9) (0.8%–11%)[f]	2 (2.6) (0.3–9%)[f]
Penicillin	4 (5.3) (1.5%–13%)[f]	4 (4.5) (1.3%–11%)[f]	2 (2.2) (0.3%–8%)[f]	2 (2.6) (0.3%–9%)[f]	2 (2.6) (0.3%–9%)[f]
Tilmicosin	Ø	Ø	1 (1.1) (0.03%–6%)[f]	Ø	Ø
Tiamulin	3 (3.9) (0.8%–11%)[f]	2 (2.7) (0.3%–8%)[f]	Ø	Ø	Ø
Tylosin	4 (5.3) (1.5%–13%)[f]	41 (46.6) (36%–58%)[f]	48 (53.3) (43%–64%)[f]	6 (7.9) (3%–16%)[f]	7 (9.2) (4%–18%)[f]
Virginiamycin	Ø	Ø	Ø	3 (3.9) (0.8%–11%)[f]	1 (1.3) (0.03%–7%)[f]

[a]Weaning phase of 76 farrow-to-finish farms
[b]Growing phase of 76 farrow-to-finish farms, 6 individual grow-to-finish farms, and 6 grow-to-finish farms within multisite operations
[c]Finishing phase of 76 farrow-to-finish farms, 6 individual grow-to-finish farms, 6 grow-to-finish farms within multisite operations, and 2 finishing farms
[d]Lactating sow phase of 76 farrow-to-finish farms
[e]Dry sow phase of 76 farrow-to-finish farms
[f]95% confidence intervals

of 1, 2, 3, 4, and 5 antibiotics was reported in 24 (31.6%), 16 (21.1%), 11 (14.5%), 13 (17.1%), and 1 (1.3%) farms, respectively. In growers, the use of 1, 2, 3, and 4 injectable antibiotics was reported in 20 (22.7%), 18 (20.5%), 13 (14.8%), and 11 (12.5%) farms, respectively. In finishers, the use of 1, 2, 3, 4, and 5 injectable antibiotics was reported in 18 (20%), 14 (15.6%), 9 (10%), 8 (8.9%), and 1 (1.1%) farms, respectively. The most commonly reported injectable antibiotics in weaners, growers, and finishers are shown in Table 5.

Discussion

According to the Canadian Pork Council's swine industry statistics for 2000 (19), Alberta had approximately 2200 swine farms in the year of the study and among those, approximately 75% had < 527 pigs per farm, 18% had 527 to 2652 pigs, and 8% had ≥ 2653 pigs. The farm size of 527 to 2652 pigs and the farm size of ≥ 2653 pigs contributed 32.6% and 59.2% of Alberta annual market pig production, respectively, or 91.8% of the total of 1 566 500 pigs produced in Alberta. More than 50% of farms in Alberta were farrow-to-finish, and most of the grow-to-finish pigs were produced in the Red Deer, Lethbridge, and Drumheller areas (20). In this study, participating herds represented approximately 25% of the annual market pig production in Alberta and were geographically representative of the major swine production areas in Alberta. Ten (7 swine only and 3 mixed animal practitioners) of the 16 veterinary practitioners in Alberta with swine clients participated in the study; they included all of the veterinarians who work exclusively with swine, thus indicating that the study had a good coverage of swine veterinary practices in Alberta. Consequently, the results should be generally representative of the larger (≥ 2000 market pigs/y) swine farms in Alberta. However, considerable caution must be exercised before attempting to generalize the findings beyond these farms, because farms producing fewer than 2000 market pigs/y were not included in the study and participating farms were not selected randomly.

No attempt was made to discriminate between 'over-the-counter' and 'prescription' antibiotic use in participating herds, thus no inferences should be made regarding prescription practices of swine veterinarians in Alberta.

The finding that the vast majority of antibiotics were used in-feed is consistent with results of previous surveys conducted in the USA and Canada (16,21–22). This practice was more frequently reported in weaners than in other categories, supporting findings of the previous studies (1,16,21) that most pigs receive antibiotics

Table 4. Reported type of antibiotic used through water in various production phases in 90 swine farms in Alberta in 2000

Type of antibiotic	Number of farms (%) using this antibiotic				
	Weaners[a]	Growers[b]	Finishers[c]	L. Sows[d]	D. Sows[e]
Dimetridazole	0	4 (4.5) (1.3%–11%)[f]	4 (4.4) (1.2%–11%)[f]	0	0
Lincomycin	0	1 (1.1) (0.03%–6%)[f]	0	0	0
Lincomycin/ spectinomycin	1 (1.3) (0.03%–7%)[f]	0	0	0	0
Neomycin/tetracycline	4 (5.3) (1.5%–13%)[f]	0	0	0	0
Oxytetracycline	1 (1.3) (0.03%–7%)[f]	0	0	0	0
Penicillin	28 (36.9) (26%–49%)[f]	9 (10.2) (5%–19%)[f]	8 (8.9) (4%–17%)[f]	1 (1.3) (0.03%–7%)[f]	3 (3.9) (0.8%–11%)[f]
Penicillin/streptomycin	4 (5.3) (1.5%–13%)[f]	0	0	0	0
Sulphamethazine	3 (3.9) (0.8%–11%)[f]	1 (1.1) (0.03%–6%)[f]	1 (1.1) (0.03%–6%)[f]	0	0
Tetracycline	3 (3.9) (0.8%–11%)[f]	4 (4.5) (1.3%–11%)[f]	4 (4.4) (1.2%–11%)[f]	0	1 (1.3) (0.03%–7%)[f]
Tiamulin	3 (3.9)[f] (0.8%–11%)[f]	0	0	0	0
Trimethoprim/sulfadoxin	3 (3.9) (0.8%–11%)[f]	1 (1.1) (0.03%–6%)[f]	0	0	0
Tylosin	0	0	0	0	0

[a]Weaning phase of 76 farrow-to-finish farms
[b]Growing phase of 76 farrow-to-finish farms, 6 individual grow-to-finish farms, and 6 grow-to-finish farms within multi-site operations
[c]Finishing phase of 76 farrow-to-finish farms, 6 individual grow-to-finish farms, 6 grow-to-finish farms within multi-site operations, and 2 finishing farms
[d]Lactating sow phase of 76 farrow-to-finish farms
[e]Dry sow phase of 76 farrow-to-finish farms
[f]95% confidence intervals

in-feed after weaning ("starter rations"), when they are most vulnerable to infectious diseases. Dunlop et al (16) surveyed 639 farms in Ontario in 1991 and found that 94% of starter feeds contained antibiotics. Bush et al (22) investigated the use of antibiotics in 895 swine operations in the USA in 2000 and found that 82.7% of farms that raised nursery pigs added antibiotics to the feed for growth promotion or disease prevention purposes.

The use of antibiotics through feed was reported in a higher percentage of farms in growers (90.9%) and finishers (80%) in the present study than in the previous surveys (16,22). The use of in-feed antibiotics in finishers was reported in approximately 30% of 639 swine operations in Ontario (16), while approximately 57% and 37.9% of the 895 USA operations with grower and finisher pigs, respectively, included antibiotics in the feed for growth promotion and disease prevention (22). Dewey et al (23) reported that large and intermediate swine farms in the USA were more likely to use feed additives in the rations than were small farms with less than 50 sows, while producers who did not use veterinary consultants were 2.1 times more likely to use feed without feed additives. In this study, all farms had $\geq$ 100 sows (mean = 525, median = 300) and all producers used veterinary consultants, suggesting that the size of participating farms and the presence of veterinary consultants may, at least partially, account for the higher percentage of farms using in-feed antibiotics.

In the current study, the use of multiple (3 or more) in-feed antibiotics was frequently reported in weaners (67.1%) and infrequently in growers (10.2%) and finishers (1.1%). Dunlop et al (16) also reported that starter rations in swine farms in Ontario often contained $>$ 1 type of antibiotic, while relatively few operations added $>$ 1 antibiotic to finisher feed. While the tetracycline/sulfamethazine/penicillin and the carbadox and lyncomycin/spectinomycin combinations were the most commonly used in-feed antibiotics in weaners in this study (Table 3), tylosin, carbadox, and furazolidone were the most commonly used in-feed antibiotics in the Ontario study (16). It is important to note that furazolidone and carbadox were removed from the Canadian market for use in swine in Canada in 1994 and 2001, respectively. Our finding that tylosin and lincomycin were the most commonly reported in-feed antibiotics in growers and finishers (Table 3) is consistent with the previous surveys (16,22).

We observed that antibiotics were less frequently administered through water than through feed (Table 1). Among the farms using antibiotics in water, occasional use of only 1 type of antibiotic was reported mostly, particularly in growers and finishers. Previous surveys

Table 5. The types of injectable antibiotics reported in various production phases in 90 swine farms in Alberta in 2000

Type of antibiotic	Number (%) of farms using this antibiotic		
	Weaners[a]	Growers[b]	Finishers[c]
Ceftiofur	10 (13.2) (6.5%–23%)[d]	4 (4.6) (1.3%–11%)[d]	9 (10) (5%–18%)[d]
Lyncomycin	3 (3.9) (0.8%–11%)[d]	3 (3.4) (0.7%–10%)[d]	3 (3.3) (0.7%–9%)[d]
Penicillin	45 (59.2) (47%–70%)[d]	50 (56.8) (46%–67%)[d]	37 (41.1) (31%–52%)[d]
Oxytetracycline	19 (25.0) (16%–36%)[d]	20 (22.7) (14%–33%)[d]	18 (20.0) (12%–30%)[d]
Trimethoprim/ sulfadoxine	28 (36.8) (26%–49%)[d]	12 (13.6) (7%–23%)[d]	13 (14.4) (8%–23%)[d]
Tylosin	7 (9.2) (4%–18%)[d]	14 (15.9) (9%–25%)[d]	10 (11.1) (5%–19%)[d]

[a]Weaning phase of 76 farrow-to-finish farms
[b]Growing phase of 76 farrow-to-finish farms, 6 individual grow-to-finish farms, and 6 grow-to-finish farms within multisite operations
[c]Finishing phase of 76 farrow-to-finish farms, 6 individual grow-to-finish farms, 6 grow-to-finish farms within multisite operations, and 2 finishing farms
[d]95% confidence intervals

(16,22) investigated the use of antibiotics in the grow-finish phase of production only and reported that 25.3% and 33.3% of farms in Ontario and the USA, respectively, included antibiotics in water in those categories, mostly for therapeutic purposes. While penicillin was the most frequent in-water antibiotic in growers and finishers in our study (Table 4), dimetridazole and tetracycline were the most frequent in-water antibiotics in the Ontario and the United States' studies, respectively (16,22).

In the United States' study (22), approximately 66.6% of the production units administered injectable antibiotics to grower and finisher pigs, primarily to treat respiratory disease. The most common strategy for all categories was to treat all pigs in a room initially and then to treat only the clinically ill pigs. In the present study, the use of injectable antibiotics was mostly reported only in sick pigs for all production phases. It has been estimated that injection site damages cost the American pork industry approximately US$40 million annually as a result of trim loss at packing plants (24). The use of injectable antibiotics was less frequently reported in growers and finishers than in weaners in this study (Table 1), possibly this type of use was avoided in certain farms to prevent injection-related side effects and tissue damage at the injection sites. Difficulties in handling larger pigs, lower morbidity, and lower individual pig observation rates in pigs of this age might also account for the less frequent use of injectable antibiotics in these categories. We observed the use of multiple injectable antibiotics in all production phases, which was consistent with findings reported in the Ontario study (16). The ranking of injectable antibiotics in growers and finishers (Table 5) was consistent with those reported in the Ontario and United States' studies (16,22).

An important limitation of the study is that information on the type of use (therapeutic or growth promotion), duration of the treatment (d) for specific types of use, and the dose were not acquired, because logistical constraints required brevity in the questionnaire. It would be beneficial to obtain this information through further studies or prospective ongoing antibiotic use monitoring, considering that in-feed antibiotic use is a practice that has been shown to be a risk factor in the development of antibiotic resistance (25). The apparent low frequency of use of critically important antibiotics for use in humans (fluoroquinolones and 3rd generation cephalosporins) is an encouraging finding from a public health perspective (3,7,11,15,25). However, the widespread and frequently reported use of penicillin and tetracycline are of public health concern, considering that both antibiotics are also used for therapeutic purposes in human medicine (1,3–7,25). Furthermore, these less expensive antibiotics have been used to protect animal health for decades (1–2), indicating a need to utilize these antibiotics more judiciously, if their benefits are to be preserved.

The most continuous use of in-feed antibiotics in weaners reported in a large percentage (96.1%) of farms was expected. We were somewhat surprised that such use was also reported in growers and finishers in 85.2% and 60.0% of farms, respectively, indicating that at least a certain percentage of the use may be for growth promotion purposes. Unfortunately, the disease status for participating farms was incomplete, because 3 practitioners declined to provide this information for 24 herds due to confidentiality reasons. This introduced a significant bias to this information and prevented analyses on potential associations between the herd antibiotic use and the herd disease status. According to the Ontario study (16), only 10% of producers thought that feeding antibiotics to healthy finishers could be profitable. When the effects of various regimens for the administration of antibiotics in feed were evaluated in multisite production systems (26), only the growth rate of nursery pigs was significantly improved, while the growth rate of finishers and the feed efficiency of nursery and finishing pigs were not significantly improved. Based on these findings, Dritz et al (26) have recommended that the use of antibiotics for growth promotion should be limited to the nursery phase in multisite production systems. Initial experiences from the European Union have indicated that the ban of antibiotic growth promoters in swine did not have any measurable impact on pig health in the growing and finishing phases, while only minor effects on performance were observed (27,28). However, negative effects were reported in weanling pigs due to the increased prevalence of postweaning diarrhea, consequently increasing therapeutic use of antibiotics (25). In the present study, no in-feed antibiotic use was reported in finishers in 18 (20%) mostly farrow-to-finish farms, indicating that this category may be a reasonable target for voluntary reduction or cessation of nontherapeutic antibiotic use. This would have an immediate positive economic impact on the swine industry, as these additives represent a substantial portion of the expenses associated with feeding pigs (26). There is also a potentially positive public health impact, as reduced antibiotic use would also reduce the pressure for selection of antibiotic-resistant bacteria (3,6,7,15,25–27). This proactive approach, along with a rigorous judicious use of antibiotics in swine (29), would allow the swine industry to preserve the benefits of existing antibiotics and to evaluate actual economic and public health impacts of

reduced antibiotic use in various production systems. However, other researchers warn that the banning of any antibiotic usage in animals based on the "precautionary principle" in the absence of a full quantitative risk assessment is likely to be wasted at best and even harmful to both animal and human health (30).

The frequency of use of antibiotics commonly administered through feed, water, and, to a lesser extent, injection differed from those observed in the previous surveys (16,21–22), indicating the importance of local industry information when assessing public health risks associated with the use of antibiotics. Antibiotic use patterns in the swine industry may vary over time. Therefore, periodically conducted surveys would be useful for monitoring patterns of antibiotic use and for understanding or anticipating the emerging antibiotic resistance patterns. Data on the use of antibiotics in swine and other food animals are essential for evaluating risks associated with the use of antibiotics in food animals, as well as for the design and planning of monitoring programs and ongoing management at the individual farm, district, regional, national, and international levels.

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